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CELLULAR REACTIONS TO FRACTIONS ISOLATED FROM TUBERCLE BACILLI

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The cellular reactions in tuberculosis form one of the striking features of the disease and it is important to note that they can be produced by the dead bacilli. In 1890, Maffucci (1) studied the effect of subcutaneous injections of dead organisms; he recorded the formation of abscesses and the subsequent death of the animals from marasmus. The next year, Koch (2), in the well-known article describing the so-called Koch phenomenon, tested the reaction to subcutaneous injections of dead, as well as living tubercle bacilli, in normal and tuberculous guinea pigs. In normal animals he also produced local abscesses with dead organisms. However, in 1890, Wyssokowitsch (3) had also studied the effects of dead tubercle bacilli, using both subcutaneous and intraperitoneal injections in rats. He found nodules of epithelioid cells and giant cells and infiltrations with neutrophilic leucocytes; that is, the production of tubercles. These observations were repeated and extended by Prudden and Hodenpyl (1891) (4) and by Prudden (1891) (5), and were then repeatedly confirmed (6–11).

The fact that dead bacilli will produce the lesions of tuberculosis is of great significance and sets the disease apart from other infections. It demonstrates that, in the actual infection, dead bacilli, as well as living, may play a rôle and gives an especial interest to the study of the tissue response to chemical fractions isolated from the organism. Proteins, carbohydrates, and lipoids all play a part in these reactions, but the lipoids alone produce tubercles. These substances have the power to stimulate the formation of the epitheloid cell and its multinuclear form, the Langhans giant cell, which together are the essential structural units of the tubercle.

While chemical analyses of the organisms give only the vaguest clues

to the complex compounds through which tubercle bacilli disintegrate in the body, it is, nevertheless, remarkable how closely the cellular reactions to certain chemical fractions reproduce the tubercle and how many of the various accessory cellular responses can be elicited by other fractions.

In the reactions to the fractions of the earliest analyses, however, it is not possible to make a sharp discrimination between the effects of proteins, carbohydrates, and lipoids. The earliest analyses of tubercle bacilli showed a high content of phosphorus and of lipoids. Hammerschlag (12) isolated material soluble in alcohol and ether and found that it made up about 27 per cent of the weight of the organisms. This material, on analysis, yielded lecithin, fat, and a certain toxic substance. On injecting the material soluble in alcohol and ether, and thus containing lipoids, the animals died with the symptoms which have since been definitely related to proteins and carbohydrates. This indicates that his material was a mixture. Hammerschlag also isolated a protein from the bacterial residue. De Schweinitz and Dorset (13) found even more lipoidal material, namely, 37 per cent.

The earliest report of a study of cellular reactions to materials from tubercle bacilli is that of Weyl (14), in 1891, who extracted the bacilli with caustic soda and obtained material which caused necrosis. Auclair (15) and Auclair and Paris (16) made a series of interesting biological studies with fractions from the tubercle bacillus. They killed the organisms with sunlight. Auclair (1897) collected the volatile substances from the organisms, responsible for the characteristic odor of the cultures, and found them toxic. With small doses there was congestion and hemorrhage in the lungs and liver; in larger doses, death. In tuberculous animals there was a rise in temperature and a rapid loss of weight. In 1898 Auclair described the serious illness of eight people from the volatile products set free while the ether extract was being filtered. Koch (1897) (2) had already called attention to the danger of grinding tubercle bacilli in open mortars. The effects of these volatile products have been repeatedly experienced by those who have worked with this organism. In the experience of the H. K Mulford Company which has handled enormous quantities of these organisms, the bovine organism liberates more of the volatile material than the other strains.

Auclair suspended the bacilli in ether and noted that two types of organisms could be demonstrated; cocci which floated in the ether and made it milky, and bacilli which sank to the bottom on standing. The

cocci, inoculated subcutaneously, gave small nodules which caseated. The bacilli, inoculated in the same way, gave lesions which were resorbed; abscesses were rare but the animals showed loss in weight. He found that ether extracted a lipoid analogous to cholesterin; alcohol, a lecithin; and chloroform, a wax. The extracts obtained from ether and chloroform were both acid-fast as were the organisms after their removal. He found that all of the lipoidal substances gave cellular reactions comparable to those produced by the bacillus. Injected under the skin, the ether-soluble material gave abscesses which caseated. With intratracheal injections, it produced pneumonia with marked caseation. The chloroform-soluble material was slower in reaction; subcutaneously it caused sclerosis, and intratracheally a reaction like tuberculous pneumonia. From these results Auclair emphasized caseation as due to lipoids from the organisms. These biological reactions to the materials extracted by Auclair were then tested in different parts of the body (17–20). Dominici and Ostrovsky (21) extracted heat-killed tubercle bacilli with water and obtained substances which, injected subcutaneously, gave tubercular tissue in the organs. They concluded that lipoids were not essential for the production of tubercles, but inasmuch as the tuberculo-phosphatide separated by Anderson, to be described later, is soluble in water, it is possible that this aqueous extract contained not only protein and polysaccharide but also some phosphatide. With this aqueous extract they also produced a general stimulation of lymphoid tissues and showed a remarkable increase in lymphoblasts (see their plate XXXIII) in the lymph follicles.

The lipoidal material which Levene (22) isolated from the tubercle bacillus was tested at the Trudeau Sanatorium and produced sterile abscesses under the skin. Morse and Stott (23) and Ray and Shipman (24) isolated lipoids from tubercle bacilli for the purpose of studying the cellular reactions to them. Morse and Stott used first ether and then boiling alcohol for the extractions. Ray and Shipman retested these methods and used also the more effective methods of Long, namely, alcohol followed by hot toluol. These lipoids were then extracted by ether and by chloroform. With the lipoids extracted by these means, both groups obtained tubercular tissue consisting of epithelioid cells, giant cells, and fibroblasts, and recognized that the formation of the tubercle may be considered as a foreign body reaction toward tuberculolipoids. Although these are important steps, it is not possible to recognize the nature of the lipoidal material extracted and their reports of the biological testing are not conclusive because they used diluting

menstrua for the tuberculo-lipoids, olive oil in the one case and sodium stearate in the other, without adequate control of their reaction.

Acid-fastness in tubercle and paratubercle bacilli was from the very beginning a factor of great interest in connection with the chemistry of the lipoids of these organisms. That certain organisms possessed the property of resistance to decolorizing both with acids and with alkalies was first noted by Neisser; this property played an important rôle in the work of Koch, and was further analyzed by Ehrlich. Koch (2) (1897) noted that if tubercle bacilli were ground in a mortar they lost this property entirely. Benians (25) and Sherman (26) confirmed this and showed that acid-fastness could be made to disappear from tubercle bacilli by simply rubbing them between two slides. These observations suggest that if the property is due to a specific substance, a simple dispersion of the substance into sufficiently fine particles is sufficient to remove the reaction. As early as 1886, Bienstock (27) and Gottstein (28) both showed that the property of acid-fastness was associated with lipoids. Auclair found that all of the lipoidal substances, as he extracted them, were acid-fast; and that, after their removal, this property was still retained by the bacilli. It is now clear that Auclair's separation of the lipoids was partial and that the complete defatting of the organisms by lipoidal solvents is extremely difficult, if not impossible without the breaking up of the bacilli. Aronson (29) found that the wax from the organism is acid-fast; this was confirmed by Borrel (30) and by Bulloch and Macleod (31). Bulloch and Macleod showed that the substance which had this property was extremely resistant to saponification and was an alcohol. In 1910, Aronson showed that saponification of the entire organism with strong alcoholic caustic potash gave an unsaponifiable material which was acid-fast. He found also that it was a higher alcohol. This has now been shown in the more recent analyses of Tamura (32), Goris (33), and Anderson and his associates (34–42). In Anderson's analysis, it has proved that the material soluble in alcohol-ether entirely lacks the property of acid-fastness after the traces of unsaponifiable material have been removed from it.

Early in the study of tubercle bacilli (1883 and 1884), Malassez and Vignal (43) suggested that there is a non-acid-fast phase of Koch's organism. They found a non-acid-fast organism in a nodule from the skin of a child who had died of tuberculous meningitis, inoculated the material into guinea pigs and recovered acid-fast bacilli. Ferran (1897) (44) then showed that non-acid-fast forms could be developed in cultures of tubercle bacilli by reducing the amount of peptone and glycerin

in the media in each succeeding transplant. The cultures which he obtained in this way contained some acid-fast organisms and were of low virulence. In 1903, Auclair (15) repeated this experiment, but by omitting the glycerine altogether, he degraded a culture into forms which were wholly non-acid-fast and avirulent. Moreover, he was unable to cause these organisms to recover their virulence. He analyzed this culture by the same methods he had been using for the acid-fast forms, obtained the same type of acid-fast lipoids as from the virulent cultures, but in less amount. Moreover, these lipoids gave the same biological reactions as those from virulent tubercle bacilli. The occurrence of non-acid-fast forms of tubercle bacilli has now been repeatedly reported (45 to 57) since their first discovery by Malassez and Vignal and Ferran. Moreover, Kahn's observations (56, 57), in which he has isolated a single acid-fast bacillus from a culture of tubercle bacilli, watched it fragment into cocci and then still further subdivide into colonies of tiny, non-acid-fast organisms, and then regenerate the original form, make conclusive proof of a non-acid-fast cycle in the life of the tubercle bacillus. This fact enhances the interest in the early observation of Auclair that the non-acid-fast form contains an acid-fast lipoid which can be demonstrated chemically.

In comparing the biological reactions to fractions from different chemical analyses, it is of the utmost importance to know the nature of the material used. In a recent extensive review, Albert-Weil (58) has made tables of many of the chemical analyses which elucidate the comparisons where possible (13–16, 22–24, 29–33, 59–68). He has presented charts which analyze the sequence of the procedures of Goris (38) and of Anderson (37–42) who have made the most extensive studies of the tuberculo-lipoids. Many factors make the comparisons of these analyses difficult: variations in the strains of the bacteria used, in the media on which they were cultivated, the age and the condition of the organisms when the analyses were made, the solvents used, and finally, as Albert-Weil has pointed out, the order in which they were applied. Perhaps the most important factor contributing to the difficulties in comparing different biological studies of the lipoids from different extractions has been brought out by Anderson, namely, that no one solvent makes a complete separation of one lipoid from the others. For example, he found that a little of the unsaponifiable acid-fast material which is soluble in chloroform came down with the entirely non-acid-fast material soluble in alcohol and ether. This means that some of the materials of the earlier analyses were probably mixtures of the lipoids

separated by Anderson. No claim of purity, in the chemical sense, is made for the products of the recent analyses, but the products have been submitted to biological testing in succeeding stages of purification. In the case of some of these products, a relatively simple and entirely constant biological reaction has been observed.

Chemical analysis of the bacteria depended from the start on devising suitable media for the growth of the organisms. This was clear to Hammerschlag (12) who made the first analysis. He said that his analysis, made not long after the discovery of the organism (1882) (2) had to await the introduction of glycerin (Nocard and Roux, 69) into the media, which made possible an adequate growth of the bacilli. Hammerschlag used glycerinated bouillon and glycerin-peptone agar. In 1892, Kühne (70) showed that tubercle bacilli could be grown on simple media without protein, and in 1894 Proskauer and Beck (71) developed what is still regarded as the simplest formula on which growth is possible, namely, ammonium carbonate, mono-potassium phosphate, magnesium sulphate, and glycerol. Luxuriant growth is necessary for chemical analysis. Armand-Delille, Mayer, Schaeffer and Terroine (72) studied the nitrogenous compounds in the media and found that the monoamino acids were the indispensable part of the peptone, while in the bouillon certain extractives (carnosin, creatin, and sarcosin) and diamino acids (argainine and histidine) were important. Kendal, Day and Walker (73) then showed that in albumen-free media containing asparagin, glycerin, and 1 per cent of either mannite or glucose, the organisms could synthesize their nitrogenous compounds from the asparagin and their fats and waxes from the glycerin and sugar. The early work of De Schweinitz and Dorset (13) had already shown that phosphorus pentoxide made up 55 per cent of the mineral content of tubercle bacilli and Baudran (74) found that tubercle bacilli use the phosphates in the media. These studies were followed by more extensive research on the metabolism of the organism and the various substances necessary for their best growth (75–80). The facts disclosed by these investigations have led to all of the synthetic media, of which the Proskauer and Beck (71), the Sauton (81), and the Long (75, 76) are examples. Only by the use of such media can one be sure that all of the complex carbohydrates, lipoids, and proteins found in the bacilli and in the fluid media on which they have been grown have been synthesized by the organisms.

A recognition of the difficulties in comparing the results of previous analyses and of the fundamental importance of using standard strains

of organisms and synthetic media without protein led to a coöperative plan by the Research Committee of the National Tuberculosis Association, William Charles White, United States Public Health Service, Chairman. This plan was to select standard strains of organisms, have them grown in large quantities on synthetic media, analyzed chemically, and have the various products tested biologically to determine how many of the factors of the disease could be reproduced. Certain standard strains of tubercle bacilli, human, bovine, and avian, together with a strain of the timothy grass bacillus and a strain cultivated from a leprous lesion, were chosen. The strains used are shown in Anderson's tables (1932). The first work was done with the human strain H-37, and then fractionation by the same procedures was made of the others. The organisms were grown by the H. K. Mulford Company on Long's synthetic media made entirely from chemicals of the same standard lot, purchased at one time. All of the cultures have been grown in pyrex glass. No pains have been spared to make the cultural conditions as uniform as possible. Moreover, the organisms have been furnished to the chemists in quantities adequate for analysis. The analyses of the lipoids and carbohydrates related to them have been made by Dr. R. J. Anderson, with the aid of Drs. E. G. Roberts, E. Chargaff, M. L. Burt, M. C. Pangborn, and N. Uyei, Sterling Chemical Laboratory, Yale University. The proteins and carbohydrates of the bacilli have been extracted by Prof. T. B. Johnson, of Yale University, with the aid of Drs. R. D. Coghill, E. B. Brown, and A. G. Renfrew. Dr. M. Heidelberger, of Columbia University, has studied the several carbohydrates from the organisms. The bacillary proteins and carbohydrates found in the media have been isolated and tested biologically by Drs. E. R. Long and F. B. Seibert, of the University of Chicago. It has been our privilege to receive some of each of these fractions for biological study. A complete bibliography of this work up to 1929 has been published (82).

Proteins from tubercle bacilli are responsible for the reactions that give the skin test (83, 84). Injected intravenously they produce the changes in the cells of the cirulating blood common to all other proteins, and give a rise of temperature in the normal animal. The tuberculous animal is remarkably sensitive to them; they give a rise in temperature or, after sufficient dosage, a rapid fall in temperature, followed by death. The cellular reaction to the proteins is mainly a proliferation of plasma cells (85). There is also damage to the endothelium of the vessels, causing hemorrhage (86). Injections of the tuberculo-polysaccharides may cause death in the tuberculous animal, and in the tissues of the

normal animal they are chemotactic and toxic to the neutrophilic leucocytes (86–89). The rise in temperature which follows the injection of polysaccharide is probably produced by an accompanying nitrogenous compound. The lipoids, on the other hand, produce a great variety of cellular reactions, reproducing many of the lesions characteristic of the disease (86–89).

CELLULAR REACTIONS TO TUBERCULO-LIPOIDS. With the abundant material furnished by the H. K. Mulford Company, Doctor Anderson (34–42) separated the lipoids of tubercle bacilli into a phosphatide, an acetone-soluble fat, and a wax. Each of these three substances gives rise to cellular reactions which are so constant and so characteristic that the material injected can be determined from the lesions.

I. *Cellular Reactions to Tuberculo-Phosphatide and Phthioic Acid. Phosphatide.* An alcohol and ether extract was first made from the bacilli. This material was then extracted with acetone, and a phosphatide which was insoluble in acetone and a fat which was soluble, were obtained. Traces of wax and fat were removed from the phosphatide and then it was further purified. The first preparation which we received from Doctor Anderson contained a few acid-fast bacilli in small clumps. After this lot, Doctor Anderson filtered the material through porcelain candles, a procedure which completely removed all of the acid-fast débris. The material is stable as far as can be judged from its biological reaction which has not changed in the material which was prepared in 1926. The phosphatide is a soft, white, granular substance. It has been examined for us under crossed Nicol prisms by Dr. R. W. G. Wyckoff of The Rockefeller Institute, who reported it to be predominantly crystalline. This, of course, does not mean chemical purity, which Doctor Anderson has not yet obtained. He found that the phosphatide from the human strain of tubercle bacilli, H-37, contained 0.36 per cent nitrogen, the state of which he has not yet determined. The phosphatides from the other acid-fast organisms contain different percentages of nitrogen, as shown in Anderson's table II (1932, 34). In 1920, Linossier (65) extracted phosphatides from tubercle bacilli which he said were sticky or crystallizable with difficulty. It may thus be concluded that the tuberculo-phosphatide is a material which can crystallize.

Another property of no less importance is that when distilled water is first applied to the phosphatide, it breaks into typical myelin figures, looking much like the material in the sheaths of fresh, medullated nerve fibers, except that it is less refractive. This property is of great interest in the biological tests, because it enables one to identify the material

after it has been phagocytized by cells. The phagocytic cells of the connective tissues have been extensively studied both in their reactions toward other damaged cells, such as leucocytes in the tissues and free red blood cells, and toward insoluble particles, such as carbon, trypan blue, and carmine. These particles, which the cells have no power to disintegrate, have been used experimentally because they can be identified. To such experimental materials may now be added the tuberculophosphatide, which can be identified by the myelin figures, if examined soon after it has been phagocytized. Moreover, one may follow to some extent the signs of the breaking up of this material within the cells, and so study the processes by which the epithelioid cell of tuberculous infections is produced.

In the fresh state, this myelin-like material stains faintly pink in neutral red; after it has been taken into cells, this characteristic stain is retained for a time. In rabbit tissues the phagocytic cells soon secrete a fluid around the myelin-like masses making them stain red in this dye and obscuring the lipoidal material. In the guinea pig, on the other hand, the cells can be found engorged and distended with entirely unchanged myelin-like masses for at least twenty-four hours after the injection of the material. The description of the processes through which the cells which have phagocytized masses of phosphatide become typical epithelioid cells, will follow. These observations, based on the identification of the phosphatide within a phagocytic cell, establish the point that the epithelioid cell represents a foreign body reaction; thus, the epithelioid cell is the final stage of a cell which has taken in, and in part disintegrated, a foreign lipoid.

The next step in these biological studies was to determine which type of cell of the connective tissues phagocytizes this material and thus gives rise to epithelioid cells. For this purpose the omentum offers great advantages. It can be separated into its two layers and spread as a film on a slide, allowing the study of the cellular changes in the living state without any distortion of the position of the cells. In no other place in the body are the cells of the connective tissues to be seen in simpler form and arrangement. In the normal omentum there are small masses of cells closely packed together, forming the so-called milk spots. The milk spots are composed of monocytes and of young connective tissue cells, somewhat less differentiated. They have basophilic cytoplasm and contain a few vacuoles and many mitochondria. There may also be the simplest type of connective tissue cell, the so-called reticular cell, in which no differentiation of granules or vacuoles

in the cytoplasm can be detected. At times there are some lymphocytes and an occasional branched clasmatocyte. Similar small collections of young connective tissue cells, true milk spots, are to be found everywhere in the connective tissues, as was shown by Möllendorff and Möllendorff (90). In the interspaces between the milk spots of the omentum are scattered branched cells of the macrophage or clasmatocyte type. They always contain some debris in their vacuoles, for they are constantly functioning with reference to substances which are passing through the omentum. These clasmatocytes of the interspaces are the cells which engorge themselves with any of the particulate materials used experimentally for the identification of these cells. They also engulf in large numbers any dead leucocytes and red cells that get free into the tissues. The mass of material which these cells will take up is important in determining their functional nature as macrophages or "big eaters," since a monocyte or even such cells as the secretory cells of the liver will take in a small amount of any of the substances with which the clasmatocyte will engorge itself.

The cells of the milk spots have more deeply basophilic cytoplasm than the clasmatocytes around them and they also have massive amounts of mitochondria, signs which are recognized as characteristic of young cells in the connective tissues. The experiments with the tuberculo-phosphatide show that it is these young cells of the milk spots which phagocytize this material in massive amounts rather than the mature clasmatocytes adjacent to them.

The tuberculo-phosphatide, injected into the peritoneal cavity as a suspension in distilled water, is taken in by the clasmatocytes, as seen in the omentum, only in small amounts, so that there is only a slight change in their activity and no change in their shape; but it is phagocytized in large amounts by the cells of the milk spots. Lymphocytes, if present, are not involved. Within three days after the injection, the young connective tissue cells and monocytes become greatly increased in size and to some extent in numbers, so that the milk spots appear swollen to the unaided eye. When these cells are stained with neutral red, it appears that the lipoidal material has been segregated within a few vacuoles in the cytoplasm. Some of these cells with highly vacuolated cytoplasm wander into the peritoneal fluid, showing that they are motile; they may also divide. In this state these cells look like clasmatocytes, but it can be shown that they were derived from the monocytes of the milk spots and not from cells which were functioning as clasmatocytes before the experiment began. They represent a stage in the transformation of monocyte into epithelioid cells.

The cells which have engorged the material appear to break it into finer and finer particles and these gradual processes we have arbitrarily described in three stages. The first stage has the largest vacuoles filled with the phosphatide, either intact or slightly changed. These vacuoles are of irregular size and shape. For convenience we shall call this stage the "phosphatide cell." In the rabbit treated with the phosphatide from the human organism H-37, this first stage lasts three or four days.

The second stage shows the cytoplasm filled with a rosette of many coarse vacuoles, uniform in size, making the fixed cell appear to have a foamy cytoplasm. We speak of this stage as the course-vacuoled epithelioid cell; it is reached in five to seven days. The typical epithelioid cell is the third stage and has a rosette of such tiny vacuoles that the cytoplasm in the fixed cell appears dense and nearly uniform in structure. This stage is reached in the second week. The rabbit cells break down the phosphatide from H-37 in a uniform manner, the vacuoles being of approximately the same size in each cell at any given time. In this regard there are marked variations with the phosphatides from the other acid-fast strains.

These different stages in the development of epithelioid cells after the injection of tuberculo-phosphatide have been illustrated in part by Sabin, Doan, and Forkner (89). They will be shown further in a forthcoming paper by Smithburn and Sabin (91) from a study of the reactions of cells to the phosphatides from the various strains of acid-fast bacteria.

With repeated intraperitoneal injections of the phosphatide there is a marked maturation of monocytes and the production of epithelioid cells in such numbers that the outlines of the milk spots become obscured. That the phosphatide from the human strain is not greatly irritating to the cells of the rabbit is shown by the fact that leucocytes are called from the vessels only after the first injection; emigration of leucocytes does not occur after succeeding injections, and those present after the first injection are quickly phagocytized by clasmatocytes and destroyed. There is an increase in undifferentiated connective tissue cells in the omentum with repeated injections of the phosphatide, but it is minimal in the rabbit. In the guinea pig this reaction is much more marked. After from ten to fourteen daily doses of the phosphatide, epithelioid cells are to be found in large masses, in circumscribed tubercles, and in tuberbular granulation tissue. Injection of phosphatide into the pleura or directly into the lung in the rabbit brings out with

especial clearness the fact that the epithelioid cell is the characteristic response to this material.

The response to the tuberculo-phosphatide is clearly the phagocytosis of this material. Other immediate reactions are minimal and consist only in a transient emigration of leucocytes from the tissues. However, important cellular reactions always develop during the second week after the introduction of this material into the tissues; the occurrence of large numbers of Langhans giant cells, the local stimulation of lymphocytes and of plasma cells, and the process of caseation.

The Langhans giant cell, as seen in supravital reaction, has the same type of rosette as the typical epithelioid cell of the third stage. Forkner (92—see also 89) has shown that this type of giant cell is a multinuclear epithelioid type, while the so-called foreign body type is produced in the reaction to foreign material by the fusion of cells, usually monocytes. In the early reaction to the phosphatide, there is a marked tendency for epithelioid cells with two nuclei to occur and by the second week there may be great numbers of Langhans giant cells; in certain animals some of the lesions of the omentum may be predominantly of epithelioid giant cells and frequently the reaction in the retrosternal lymph nodes is made up entirely of these types. The epithelioid giant cell never shows the first stage of the phagocytosis of the lipoid and almost never the stage of the coarse vacuoles. It is a typical epithelioid cell which has become multinucleated. These giant cells show no especial relationship to caseation.

In every experiment in which as much as two weeks have elapsed after the injection of the phosphatide, the tissues have been found with considerable infiltrations of lymphocytes. In some cases the tubercles have had complete capsules of lymphocytes like those to be seen in the actual infection. The areas in which giant cells predominate are likely to show many lymphocytes as well. At present we are unable to analyze this late association of lymphocytes with epithelioid cells in the reaction to the phosphatide, but we have seen no evidence that it is an immediate and direct effect of the phosphatide. In this connection the observations of Dominici and Ostrovsky must be recalled (21).

The epithelioid cell may remain for long periods of time; some have been found six and one-half months after the injection of the lipoid, but in every experiment with any of the phosphatides from the acid-fast bacilli, we have found considerable amounts of caseation during the second week. We have not designated any nodule caseous unless it showed clearly a border of intact epithelioid cells around a center of

necrotic tissue; indeed, in many of the caseous nodules the outlines of the dead epithelioid cells are still present in the center, surrounded by leucocytes. It is our view that caseation is the end stage of the epithelioid cell, the infiltration of the leucocytes being secondary to the death of these cells. Thus the so-called "caseation fraction" of Auclair would be interpreted by us as a material which had produced epithelioid cells. Whether or not the death of epithelioid cells is hastened by tuberculo-protein is being investigated. Caseation is more extensive in the tissues of the guinea pig than in those of the rabbit, after the phosphatide from H-37.

In general the reactions to the phosphatide from the bovine and avian strains of tubercle bacilli, and the timothy grass bacillus and the lepra strain are like, in kind, to those from the human tubercle bacillus but differ in the time which the cells require to break down the lipoid. The reaction to the avian phosphatide is most like that to the human. It differs in that the intracellular dispersion of the material is a little slower, so that at the end of ten days, the epithelioid cells, which are present in massive numbers, are largely in the second stage rather than in the third, that is to say, they are epithelioid cells with coarse vacuoles. There may even be a few cells still in the first stage. There may be only a few giant cells, or, in other animals, they may occur in massive numbers. There is a marked tendency for these giant cells to be of complex type. Instead of being typical epithelioid giant cells of moderate size and with peripheral arrangement of nuclei, these cells are large, have one or more wide cytoplasmic areas corresponding to the rosette of the epithelioid cell, and great masses of nuclei closely packed together either in the center or at one end. They may have resulted from the fusion of several Langhans giant cells. In the reaction to this phosphatide there are quite extensive masses of plasma cells and the same infiltration with lymphocytes as with the material from the human tubercle bacillus. Caseation is marked in the areas of epithelioid cells, whether in the subcutaneous nodules or in the omentum, as well as in the lymph nodes draining these areas.

The reactions of the cells of the rabbit toward the phosphatide from the bovine organism and from the timothy grass bacillus are much alike; these materials are broken down more slowly than the phosphatide from H-37 and from the avian bacillus. In the lesions which have been produced by the bovine phosphatide, there are marked variations in the epithelioid cells, even after two weeks; in some areas these cells are of the third stage with enormous numbers of giant cells; in other areas

the epithelioid cells are in all three stages, showing an irregular breaking down of the phosphatide. There may be an increase in undifferentiated connective tissue cells, and more infiltration with leucocytes, lymphocytes, and plasma cells than after the phosphatide from H-37.

Two weeks after the injection of the phosphatide from the timothy grass bacillus there seems to be less formation of epithelioid cells but this is only because all of the large nodules have become caseous; the diffuse reaction of intact epithelioid cells shows the same mixed types as after the bovine phosphatide. After ten doses of the former, many of the epithelioid cells are still in the second stage and some even in the first. The material seems to be more irritating than any of the other phosphatides, for even after one intraperitoneal injection there are several abscesses in the omentum; and in the later reaction there are many leucocytes scattered in the tissues and phagocytized by clasmatocytes. There are also many plasma cells.

The study of the phosphatide from the lepra organism is incomplete since only small amounts have been available. The material is more irritating and the tissues show more of an increase in connective tissue cells. Also there has been much more variation in the reaction of different animals. In one experiment there was a considerable diffuse reaction of very complex epithelioid cells, a few of them being in small clumps or tubercles. In the regional lymph nodes there was, however, a massive reaction of epithelioid giant cells, a few of them being the compound types. There was also some caseation and a general infiltration of the tissues with leucocytes and many clumps of plasma cells. In another animal the reaction was almost wholly of leucocytes, enormous numbers of them having been called from the vessels and phagocytized by clasmatocytes.

From this study with the phosphatides of the acid-fast organisms, we have designated the reaction of the material toward the production of epithelioid cells and their multinuclear form, the Langhans giant cells, as the specific reaction toward tuberculo-lipoid. By this use of the term "specific reaction" is meant to imply that emphasis should be put on the epithelioid cell, either in mono- or multinucleated form, as the one structure which is essential in tubercles or in tubercular infiltrations or in tubercular granulation tissue. The phosphatides from all of the acid-fast organisms included in this study produce epithelioid cells and Langhans giant cells which go on to caseation. Caseation is, therefore, a part of the specific reaction.

All other reactions, such as the calling of leucocytes from the blood

vessels, their phagocytosis and destruction by clasmatocytes, the development of plasma cells, the increase in lymphocytes, in young connective tissue cells, in fibroblasts, and the development of new blood vessels, we have termed non-specific. On this basis, it is clear that the phosphatide from the human strain of tubercle bacilli gives the more specific response, while all of the other phosphatides give more non-specific reaction as well, the reactions to the phosphatide from the timothy grass bacillus and the lepra strain being the more mixed.

Of the various controls only one material so far tested acts just like the tuberculo-phosphatide, namely, lecithin. A quantity of this substance was prepared for us from the brain, by Dr. P. A. Levene. The lecithin is phagocytized by the same mononuclear cells as the tuberculo-phosphatide and produces epithelioid cells and giant cells in massive tubercles. In the original report (89) it was stated that only a small reaction was found in the omentum. Subsequent studies of sections from many places in the peritoneal cavity have shown an extensive reaction elsewhere. A dilauryl acetic acid containing the same number of carbon atoms as the phthioic acid, namely 26, synthetized for us by Prof. Roger Adams, Department of Chemistry, University of Illinois, is also phagocytized by the cells in the milk spots rather than by clasmatocytes, but this material does not change these cells into epithelioid types in the same period of time.

An important property of the tuberculo-phosphatide is that it acts as an antigen. This reaction was first described for alcoholic extracts of tubercle bacilli by Meyer (93); it has been studied for methyl extracts by Boquet and Nègre (94). Pinner (95) showed the phosphatide by Anderson to be antigenic, and further studies of this reaction have been made by Doan (96), and Doan and Moore (97).

Phthioic acid. All of the primary divisions of the lipoids, the phosphatide, the waxes, and the acetone-soluble material yielded on analysis certain hitherto unknown, saturated fatty acids of high molecular weight. A fatty acid designated Fatty Acid I, from the phosphatide A-3, of the human strain of organisms, was tested first in the rabbit. All of the tendency of the phosphatide toward the production of epithelioid cells was carried over into this acid; and this acid produced as great an amount of epithelioid cells as the original phosphatide (see table 1, in 88). Tubercles surrounded by lymphocytes were characteristic of the reaction. It was, however, more irritating than the phosphatide, there was a greater increase in connective tissue cells, and there were large abscesses. Typical caseation, in the sense we have defined

it, was present only in small foci. The cellular reactions simulated those of the disease more closely than those after the phosphatide. On further analysis of Fatty Acid I, Doctor Anderson obtained a hitherto unknown fatty acid of high molecular weight, which has been found in the biological test to be the only substance from the original acid producing a response of tubercular tissue. Doctor Anderson therefore named it phthioic acid. The empirical formula of this acid is $C_{26}H_{52}O_2$. The phosphatide also yielded glycerophosphoric, oleic, and palmitic acids. Each of these was tested by intraperitoneal injections and gave leucocytes both free and in clasmatocytes, some increase in blood vessels, and a non-specific thickening of the omentum in varying degrees.

The phthioic acid was a heavy oil and proved much too irritating to be used undiluted; it caused necrosis and gave very complex cellular responses. In spite of this complexity of these reactions, it could be made out that there was a marked maturation of monocytes. It was necessary, therefore, to find a bland oil for a diluting menstruum; olive oil was tried and rejected on account of its extreme irritation of the connective tissues. Mineral oil was chosen because, though not inert, it gave only a moderate increase in fibroblasts and a minimal production of epithelioid cells. Diluted in mineral oil, the phthioic acid from the phosphatide A-3 gave a diffuse reaction of epithelioids as well as typical small tubercles, like those from the phosphatide and closely simulating those of the disease. None of the highly vacuolated types of cells produced by the phagocytosis of the complex lipoid appeared with the fatty acid; the epithelioid cells were all of the typical third stage. These results may be interpreted as indicating that the original phosphatide in these experiments was phagocytized by cells and reduced to some state comparable to the fatty acid. In the one case the degradation of the material was done within the cell and in the other in the test tube. However, it is probable that in the disintegration of the bacilli that occurs in the actual infection, the phagocytosis of the material by cells takes place when the substances are in the more complex molecule. Interesting evidence bearing on this point is to be found in the work of Bickford (98) who has shown that there is a specific type of early epithelioid cell in the meninges which is produced by the injection of living or dead tubercle bacilli or tuberculo-phosphatide.

The fatty acids from the purified and soft waxes also produced typical tubercles in varying amounts, but not as massive reactions as that from the fatty acid from the phosphatides. The acetone-soluble material contained more of these fatty acids than the other lipoids. Doctor

Anderson separated these fatty acids and found an isomer of stearic acid, which he called tuberculo-stearic acid, and phthioic acid. The former was irritating but did not produce tubercular tissue.

The acetone-soluble material yielded enough phthioic acid for fractionation and Doctor Anderson separated the acid from this source into a dextro- and a levorotatory form. The specific activity toward the production of epithelioid cells was carried only in the dextrorotatory acid. The response to both of these acids is complex and the actual increase in new tissue is fully as great with the levorotatory acid. Introduced intraperitoneally in mineral oil, the levorotatory form gives a marked increase in thickness of the omentum and subperitoneal tissues. There is an increase in young connective tissue cells, an infiltration of the tissues with leucocytes, much phagocytosis of them by clasmatocytes, an increase in lymphocytes and plasma cells, together with the development of many monocytes but practically no epithelioid cells. After the dextrorotatory acid, on the other hand, there is also a marked production of epithelioid cells, and a few epithelioid giant cells; there is relatively little tendency toward the production of these cells in clumps or tubercles and we have not found these cells surrounded with lymphocytes. There is the same complex non-specific reaction toward leucocytes and plasma cells with the dextrorotatory acid as with the levorotatory, but the presence of the epithelioids in the one case makes such a difference in the appearance of the tissues that the type of acid used can be determined from the tissues without reference to records.

The question has been raised by Boissevain and Ryder (99) as to whether the alcohol-ether soluble material isolated by Anderson is really a phosphatide or merely debris of bacilli, and therefore whether the biological reactions which we have recorded with this material are necessarily associated with lipoids. The fact that dead tubercle bacilli also produce tubercles makes this question pertinent. Acid-fast debris has not been present in any of the phosphatides prepared by Anderson after the first lot; the filtering through candles removed all demonstrable acid-fast masses. Therefore the question at issue is the presence of non-acid-fast debris. It is possible that this question cannot be wholly settled until the nature of the nitrogen present in the phosphatide has been determined and until complete purification of the material has been accomplished, but the evidence up to the present time in favor of the hypothesis that the epithelioid cell is a foreign body reaction to tuberculo-lipoid is as follows. Epithelioid cells have been produced only by extracts which have been obtained by lipoidal solvents, not by

tuberculo-proteins or polysaccharides; thus if the epithelioid cell can be produced chemically at all, it must be by lipoids. The predominantly crystalline nature of the dry phosphatide and its transformation immediately on wetting into myelin figures are against the view that this material is amorphous debris. The identification of the phagocytosis of this myelin-like material by cells in a definite location, namely, in the milk spots of the omentum, so that the reaction can be followed into typical epithelioid cells, gives evidence that the epithelioid cell represents a foreign body reaction toward a lipoid. The production of the epithelioid cell in the same location by pure lecithin also suggests that the epithelioid type may be a form of reaction toward ingested lipoids.

Doctor Boissevain has also brought up the interesting question of the discrepancy between the amount of epithelioid tissue produced by dead tubercle bacilli and the corresponding amount of phosphatide on the basis of its being 6 per cent by weight of the organisms. These two quantitative reactions cannot be compared for the phosphatide contains only a part of the specifically active lipoid. Doctor Anderson has shown that all of the lipoids extracted by solvents, the alcohol-soluble, chloroform-soluble, and the acetone-soluble material, contain phthioic acid; moreover, lipoidal solvents do not remove all of these substances, for the so-called "defatted bacilli" are still acid-fast and produce epithelioid cells, in less amount, however, than intact dead bacilli. The total lipoid can only be extracted by methods which completely disintegrate the bacilli. If the total lipoidal material were available, comparative tests between the amount of the epithelioid reaction to this material and dead bacilli could be made. For such tests, however, the intraperitoneal route is not as good as the subcutaneous on account of the wide dispersion of the reaction in the peritoneal cavity. In this area the reaction is produced not only in the omentum but under the serosal lining of the body-wall, the wall of the intestine, in the mesentery, the diaphragm, in the capsules of the liver and spleen, and around the reproductive organs. The cellular reactions are also reflected by the cells free in the peritoneal fluid and always appear in the retrosternal lymph nodes which drain the peritoneal cavity. They are not found in the mesenteric lymph nodes unless the bowel wall has been involved. The value of the intraperitoneal route is the opportunity to follow the cellular reactions in living tissue without any distortion in the arrangement of the cells. In subcutaneous tests, however, all of the cellular reactions are in a restricted area except that which is to be found in the regional lymph nodes.

There are, however, certain quantitative discrepancies in our results which cannot be explained at present. In all of the tests with the more purified forms of the phthioic acid there has been a marked loss in the amount of specific activity from the reaction of the corresponding amount of the original phosphatide and the mixture of fatty acids extracted from the phosphatide. Moreover, the acetone-soluble material which contained more of the phthioic acid than the phosphatide itself shows little tendency for the epithelioid cells to be in large masses or tubercles. The question as to whether these phenomena are due to any accessory factors cannot be answered at the present time.

II. *Cellular Reactions to the Unsaponifiable Wax.* Doctor Anderson considers the so-called waxes to be very complex phosphatides. From them he isolated an unsaponifiable material having the property of acid-fastness which discriminates the group of the tubercle and paratubercle bacilli. Doctor Anderson has found this material to be a higher alcohol with the formula of $C_{94}H_{188}O_4$ and has discussed the relationship of this material to the higher alcohols of other analyses. We have had the privilege of studying also a similar acid-fast material prepared from tubercle bacilli by Dr. P. A. Levene. Both specimens were completely insoluble in water and had therefore to be suspended in mineral oil. In this menstruum they produced identical reactions, namely, a marked production of young connective tissue cells, both diffusely and in small clumps. There has been no sign that this material is phagocytized by any of the cells. The new cells which appear after the injection of this material are round or oval and of about the size of monocytes. Little differentiation can be made out in the cytoplasm which is slightly basophilic. However, the cytoplasm is so delicate that it cannot be made out at all in sections, so that the clumps or pseudo-tubercles of them look like masses of large nuclei without cytoplasmic outlines around them. There are considerable numbers of leucocytes scattered diffusely and infiltrating the pseudo-tubercles. Similar clumps of cells characterize the reaction to the levorotatory phthioic acid. Thus the cellular reaction to the unsaponifiable alcohol is a double one: undifferentiated connective tissue cells and leucocytes.

III. *Cellular Reactions to the Acetone-Soluble Fat.* By far the most complex cellular reactions are produced by the acetone-soluble fat. We have tested this material from the human, bovine, and avian tubercle bacilli and from the timothy grass bacillus. The supravital studies show that every type of connective tissue cell has been stimulated. The analysis of this material by Doctor Anderson shows that it is a complex

mixture of fatty acids, butyric, palmitic, stearic, cerotic, linoleic, linolenic, tuberculo-stearic, and phthioic acids. Corresponding to the phthioic acid there is a diffuse reaction of epithelioid cells; besides this, there is a general infiltration of the tissues with leucocytes, and many of them are in clasmatocytes; there is also a great increase in undifferentiated connective tissue cells, fibroblasts, lymphocytes, plasma cells, and a marked increase in new blood vessels with hemorrhage. The diffuse character of the epithelioid reaction may be associated with the intensity of these non-specific reactions. Further studies with the material from the various acid-fast strains of organisms are necessary in order to analyze the complexity of these reactions, which may be due in part to the high acidity of the material. As a control for this part of the lipoid, we have tested the acetone-soluble lipoid prepared from the streptococcus by Doctor Heidelberger. It appears to be the only type of lipoid in this organism; it is present only in small amounts in these organisms but gives very complex cellular responses with, however, no epithelioid cells.

From these studies of cellular reactions toward tuberculo-lipoids, the mass of evidence seems to indicate that they are remarkable stimulants of the cells of the connective tissues. Some of this material, such as the phosphatide, seems to act as a foreign body and produces effects through being phagocytized by cells; other materials, such as the unsaponifiable higher alcohol, act as a stimulant without being phagocytized; and the third lipoid, the acetone-soluble fat, is an extremely complex irritant. Reason for the cellular lesions in tuberculosis can be found in all these reactions. The view that the epithelioid cell is the result of the phagocytosis of a tuberculo-lipoid by monocytes and young connective tissue cells is in agreement with the view that the tubercle in the disease arises locally from fixed connective tissue cells.

Cellular reactions to polysaccharides. Polysaccharides obtained from the lipoids by Doctor Anderson and from the whole bacillus by Doctor Heidelberger have been tested on the cells of the connective tissues. All of these materials give the same effect, namely, the calling of leucocytes from vessels and the damaging of them so that they are quickly phagocytized by clasmatocytes. This is a constant reaction and occurs regardless of the number of injections. There is no substance tested from the tubercle bacillus that did not call leucocytes from the vessels and in some instances this has been an extreme reaction in response to some of the lipoidal fractions, as for example, the acetone-soluble fat. However, this reaction is so consistently found with all

of the polysaccharides as to raise the issue as to whether the material in the other fractions which so damages the leucocytes that they are quickly taken in by clasmatocytes may not be the content of carbohydrate.

CELLULAR REACTIONS TO PROTEIN. It was shown by Doctor Miller (85) that the various preparations of protein obtained from the tubercle bacilli all have a remarkable power toward the production of plasma cells. With repeated injections of the protein he could follow the complete life cycle of these cells, producing its youngest stages, the well-known mature, Marschalkow type, and the final stage, the so-called Russell body cell. Of all of the materials from the tubercle bacillus, the tuberculo-protein gives plasma cells in the most massive amounts. They are found, however, in considerable numbers after repeated injections of the polysaccharide; and, as has been stated, they are increased over normal numbers after the phosphatide, especially from the organisms other than the human. It is thus interesting to speculate as to whether a response of plasma cells to polysaccharide or lipoid may be a biological test of the presence of some nitrogenous compound.

SUMMARY

In these studies on the cellular reactions to chemical fractions from the tubercle bacillus, it has been shown that there are three different types of complex lipoids in the organism which can be discriminated by the cellular reactions they produce.

The phosphatide reproduces the tubercle; it is phagocytized by certain cells of the connective tissues, namely, by monocytes, and partially degraded within them, thereby forming the epithelioid cell. The fact that this material is phagocytized, and good evidence of this is obtained by seeing the characteristic myelin figures of the original material within the living cell, goes far to indicate that it is the substance itself and not some contaminating impurity which is responsible for this action.

The only constituent of this phosphatide which can produce this reaction is a saturated fatty acid of high molecular weight, phthioic acid, of the formula $C_{26}H_{52}O_2$. All of the other lipoids of the original fractionation, the wax and the acetone-soluble fat, contain also some of the phthioic acid, and therefore possess varying degrees of specific biological activity.

The waxes contain phthioic acid and an unsaponifiable residue which

is a higher alcohol, $C_{94}H_{188}O_4$. This unsaponifiable base of the wax does not seem to be phagocytized by cells, but in spite of its insolubility in water, when injected in an oil, acts as a remarkable stimulant toward the production of undifferentiated connective tissue cells. It is always irritating and calls leucocytes from the vessels. These two exceedingly complex phosphatides, the tuberculo-phosphatide and the waxes, then, may be considered as the types of lipoidal substances especially characteristic of the acid-fast strains of the organism, the phosphatide and the phthioic acid being responsible for the epithelioid cell—so prominent a factor in the cellular reactions of the disease, while the unsaponifiable material is responsible for the acid-fastness of the bacillus.

The especial interest of the acetone-soluble material is that it may be more like the lipoids of other strains of organisms; the extremely varied cellular reactions which it produces may be due to the fact that it is a complex mixture of fatty acids.

The characteristic cellular response to tuberculo-protein is the plasma cell. The tuberculo-polysaccharides are chemotactic and toxic to neutrophilic leucocytes.

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THE CHEMISTRY OF THE LIPOIDS OF TUBERCLE BACILLI

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Ever since Koch (1) discovered the tubercle bacillus and determined its relation to tuberculosis the organism has been the object of more intensive chemical and biological investigations than any of the other bacteria. In the first chemical study, published by Hammerschlag in 1888 (2), it was found that from 26 to 28 per cent of the dried bacillus could be extracted with alcohol and ether. These results have been fully corroberated by later investigations and it is now recognized that from 20 to 40 per cent of the dried cells represent lipoid compounds. During the past 40 years there has developed such an extensive literature dealing with the lipoids of the tubercle bacillus that it now forms one of the most impressive chapters in the experimental study of the disease.

The lipoids represent an extremely important fraction and some of the chief biological characteristics of the bacillus are associated with the lipoid constituents. It is believed that the bacillus is surrounded by a difficultly permeable membrane consisting of fatty and waxy material which in part serves to protect the organism from destructive influences in the host and the waxy coating is apparently responsible for the acid-fastness. Specific antigenic properties have been attributed to certain of the lipoid fractions and it has been known for some time that these components exert a peculiar stimulus on abnormal cell development in animals.

The cellular reactions caused by the bacillary lipoids have been carefully studied during the past few years in Doctor Sabin's laboratory (3) and it has been shown in a series of brilliant investigations that all of the lipoid fractions act as maturation factors for monocytes, epithelioid cells and giant cells resulting in the formation of tubercular tissue. This effect has been traced to certain new and peculiar optically active fatty acids of high molecular weight which have been isolated from the lipoids after saponification.

From the chemical point of view the total lipoids of the tubercle bacillus represent an exceedingly interesting and complex mixture consisting

of phosphatide, acetone-soluble fat and wax. The preliminary separation into the groups mentioned above was possible because the lipoid fractions possess distinct differences in solubility in organic solvents. All of the fractions that we have thus isolated have been found to differ in composition from analogous compounds of plant or animal origin which belong to these general groups.

An excellent review of the older chemical work is given in the paper by Goris (4) and a similar treatment will be found in the book by Calmette (5). The chemistry of the tubercle bacillus and related acid-fast bacteria, as known up to 1923, has been fully described by Long (6) and the comprehensive monograph by Albert-Weil (7) includes a complete bibliography of recent work. The literature dealing with the biological reactions of the lipoids has been considered by Sabin and collaborators (3) while serological experiments have been reviewed by Pinner (8). The literature dealing with the chemistry of the bacterial lipoids has also been considered in the various papers which have been published from this laboratory. It seems unnecessary therefore to include a detailed study of the older literature at this time because the purpose of the present paper is to discuss the newer developments in the lipoid chemistry of the tubercle bacillus.

METHODS OF PROCEDURE. *Cultivation and collection of the bacteria.* In this review the descriptive portion concerns itself with the methods employed in the work on the lipoids of the human tubercle bacillus, strain H-37. Similar or identical methods were used in the studies on the avian and bovine tubercle bacilli, the timothy bacillus and the B-leprae. The tables contain all of the experimental data from all of the acid-fast bacteria which are available up to the present time.

The cultures, 2000 in number, were grown in 1 liter pyrex bottles, each bottle containing about 200 cc. of the Long synthetic medium (9), at the laboratories of H. K. Mulford Company, Glenolden, Pa. The growth was luxuriant and after an incubation period of six weeks the living bacteria were filtered off on large Buchner funnels, washed with water, and immediately placed in a mixture of equal parts of alcohol and ether contained in 5 gallon pyrex bottles, the bacilli from about 450 cultures being added to each bottle. After being securely stoppered the bottles were shaken until the bacteria were uniformly dispersed. The containers were then transported to the Sterling Chemistry Laboratory at New Haven.

During the filtration of the bacteria, streams of carbon dioxide were passed over the Buchner funnels. The solvents were always freshly

distilled before being used and the alcohol had been distilled over potassium hydroxide. All solvents were saturated with carbon dioxide and all vessels employed were filled with carbon dioxide before any material was introduced into them.

The extractions were conducted at room temperature. The extracts were concentrated at a temperature not exceeding 35° and during vacuum distillations carbon dioxide was admitted through the capillary tubes. By taking these precautions it was hoped to prevent changes due to oxidation or to high temperature and to secure the lipoids in a condition as similar as possible to that in which they existed in the living cells.

Extraction of the bacterial lipoids. The bacilli were extracted with the alcohol-ether mixture for a period of 4 weeks; the containers being frequently shaken. The bacterial cells settled rapidly and the perfectly clear, slightly amber colored extract was siphoned off under carbon dioxide pressure. The cell residues were filtered on Buchner funnels and washed with ether. The alcohol-ether soluble constituents were isolated as will be described later.

Extraction with chloroform. The cell residues were next extracted with chloroform in 5 gallon pyrex bottles, filtered and treated a second time with chloroform. The extracts were filtered and the solvent was removed by distillation when a yellow waxy residue was obtained which weighed 427 grams. This chloroform soluble material consisted of crude wax.

The extracted bacterial cells were dried in vacuo at a low temperature and reserved for the future study of water-soluble constituents such as proteins, carbohydrates and nucleic acid.

EXAMINATION OF THE ALCOHOL-ETHER EXTRACT: *Separation of the acetone soluble fat.* The filtered extract was warmed to about 35° and the ether was removed by a current of carbon dioxide. The alcohol was then distilled off under reduced pressure leaving an aqueous suspension of lipoid material and this mixture was shaken repeatedly with ether until the lipoids were dissolved.

The aqueous solution which now remained served for the isolation of water-soluble constituents and especially for the preparation of a polysaccharide.

The ethereal extract, containing all of the alcohol-ether soluble lipoids, was dried with sodium sulfate, filtered, and concentrated at a low temperature by means of a current of carbon dioxide to a volume of about 500 cc. An equal volume of acetone was added when a dense sticky mass separated on the bottom of the flask. The clear supernatant

liquid was decanted and the precipitate was washed several times with acetone. The washings and the decanted mother liquor were united, concentrated, and mixed with more acetone when a further quantity of a sticky precipitate separated. After the mixture had been cooled in ice water the clear supernatant solution was decanted and the precipitate was washed with cold acetone. The acetone-insoluble material obtained in this manner, consisting of crude phosphatide and a small amount of wax, was purified as described below.

The acetone mother liquors and washings were united; the solvent was distilled off under reduced pressure and the residue was dried in a current of carbon dioxide. This residual material which was soluble in cold acetone represents the so-called acetone-soluble fat. It was a dark brown oil when warm but on cooling to room temperature it formed a soft salve-like mass and it possessed a peculiar not unpleasant perfume-like odor. This fraction weighed 240 grams and its chemical composition will be described in a subsequent section.

Purification of the phosphatide fraction. The crude phosphatide obtained as mentioned above was dissolved in 200 cc. of ether giving a nearly clear, amber colored, solution. An equal volume of acetone was added when a dense gummy precipitate separated on the bottom of the flask. The supernatant liquid was decanted and the precipitate was washed with acetone. The substance was precipitated in this manner 5 times but the last precipitation was done by pouring the ethereal solution into 750 cc. of ice-cold acetone when the phosphatide separated in the form of a faintly yellowish amorphous powder which after it had been dried in vacuo weighed 148.5 grams.

The mother liquors were cooled in a freezing mixture when a large amount of an amorphous precipitate separated which was filtered off and washed with acetone. The filtrate was concentrated by distillation until most of the ether had been removed. The solution was now cooled in a freezing mixture when a further quantity of precipitate was obtained, which was filtered, washed with acetone and dried. The two precipitates were combined giving 104.6 grams of impure phosphatide.

The acetone mother liquor was concentrated to dryness when a small quantity of fatty material was obtained which was combined with the acetone-soluble fat.

The total crude phosphatide obtained as mentioned above amounted to 253.1 grams. The two fractions were purified separately by repeated precipitation from ether with acetone until the composition was constant.

The mother liquors obtained in the last purifications yielded 58.6 grams of a white amorphous powder which was practically free from phosphorus and nitrogen. This substance was similar to the material which has been designated unsaponifiable wax.

Summary of the separation of the lipoid fractions. The methods of extraction, separation and purification of the bacterial lipoids into fractions consisting of phosphatide, acetone-soluble fat, and wax have become standardized in this laboratory. With very slight modifications

TABLE 1

Lipoid fractions from acid-fast bacteria

	TYPE OF ORGANISM									
	Human H-37		Avian # 531		Bovine # 1698		Timothy # 02145		Leprosy # 370	
	Number of cultures									
	2,000		2,000		1,700		1,600		3,000	
	grams	*per cent*	*grams*	*per cent*	*grams*	*per cent*	*grams*	*per cent*	*grams*	*per cent*
Phosphatide	253.1	6.54	79.7	2.26	60.5	1.55	18.7	0.59	100.5	2.20
Acetone-soluble fat	240.0	6.20	77.3	2.19	131.7	3.34	87.4	2.75	289.5	6.47
Chloroform-soluble wax	427.0	11.03	379.5	10.79	336.0	8.52	158.4	4.98	444.8	9.98
Total lipoids	920.1	23.78	538.5	15.26	528.2	13.40	264.5	8.37	834.6	18.7
Dry bacillary residue	2902.0	75.01	2942.7	83.71	3370.1	85.50	2783.1	87.70	3389.8	80.38
Dry bacterial matter per culture	1.928		1.757		2.318		1.982		1.488	

these methods have been applied in the studies of the avian (11) and bovine (12) tubercle bacilli, the timothy (13) bacillus and B-leprae (14). It should be mentioned that this preliminary separation into more or less homogeneous fractions has been found to be very useful not only in the chemical investigations but also in the biological experiments.

The first extracts obtained from the human bacillus were only filtered through paper and not through porcelain candles because the necessary apparatus was not available at that time. All of the other bacterial extracts have been filtered not only through paper but also through Chamberland candles under carbon dioxide pressure.

The quantities of lipoids obtained from acid-fast bacteria which have been studied in this laboratory are given in table 1.

In undertaking this work involving a systematic fractionation of the lipoids followed by careful chemical analyses of the different fractions, it was hoped that the results would reveal certain differences in composition that would help to explain variations in virulence of the pathogenic bacilli. It is evident from the data presented in table 1 that qualitatively the results are similar but the quantitative relations of the different fractions show very great variations.

Analysis of the phosphatide (15). In the purification of the phosphatide several top and bottom fractions were isolated and analyzed. The composition of these fractions was practically identical, however, as shown by the following analytical results:

a. Found, P, 2.14; N, 0.40 per cent.
b. Found, P, 2.30; N, 0.36 per cent.
c. Found, P, 2.03; N, 0.35 per cent.

Properties of the phosphatide. When the phosphatide was precipitated by adding acetone to the ethereal solution it separated as a dense sticky mass which collected and adhered to the bottom of the flask. On the other hand when an ethereal solution of the phosphatide was poured into ice-cold acetone, it separated as an amorphous solid which after it had been filtered off, washed with acetone, and dried formed a nearly white or straw-colored non-hygroscopic powder.

When heated in a capillary tube the phosphatide darkens at about 200° and it melts to a dark-brown liquid at 210°. It is easily soluble in ether, chloroform or benzene and it can be precipitated almost quantitatively by acetone, alcohol or methyl alcohol. When rubbed up with water it forms an opalescent solution which on sufficient dilution becomes perfectly clear. The concentrated opalescent solutions are coagulated or precipitated by the addition of acids or salts. The aqueous solution shows a decided acid reaction on litmus paper. The aqueous suspension when boiled with Fehling's solution gives a perfectly clear solution and no reduction is obtained but after the suspension has been boiled for some time with a mineral acid it gives a heavy reduction with Fehling's solution. It is evident therefore that the substance contains a polysaccharide which yields reducing sugars on hydrolysis.

The physical properties of the phosphatide, especially its ready dispersion in water, makes it easily available for biological experiments. This is one reason perhaps why this fraction has been more thoroughly studied by biologists and immunologists than the other lipoids which

cannot be dispersed in water. The work described by Sabin and collaborators (3) on the effect of the phosphatide on the maturation of monocytes, epithelioid cells and giant cells and the immunological studies by Pinner (16) and by Doan (17) was done with the purified preparation that has just been described. Subsequently similar experiments have been made with phosphatide preparations isolated in the Mulford laboratories but the results were essentially identical.

The methods of isolation and purification outlined above have been applied in the study of the phosphatides from other acid-fast bacteria. All of these products have been found to be similar in composition and properties and to give analogous biological reactions. The chemical composition as determined by analyses before and after hydrolysis is shown in table 2.

TABLE 2

Composition of bacterial phosphatides

	HUMAN	AVIAN	BOVINE	TIMOTHY	LEPROSY
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Phosphorus	2.30	2.18	1.87	2.80	1.75
Nitrogen	0.36	0.48	1.00	0.22	Trace
Melting point	210°	210°	208°	190°	231°
Ether soluble	66–67	55–56	57–58	60.0	62.2
Water soluble	33–34	46–47	43–44	40.0	38.0

No claim to chemical purity in the ordinary sense can be made for the phosphatides. It is fully realized that it is impossible to attain a state of complete chemical purity of these compounds by the methods that have been used in their purification but we believe that extraneous admixtures of fat and wax have been removed.

The presence of phosphatide or a lecithin-like substance in the fat from the tubercle bacillus was indicated by Hammerschlag (2) who reported that the ether extract contained phosphorus. Similar observations have been reported by Kresling (18), Baudran (19), Auclair and Paris (20), Aronson (21), Bürger (22), and by Goris (4). Tamura (23) isolated a phosphatide from an alcoholic extract of the tubercle bacillus which he regarded as a diaminomonophosphatide. Agulhon and Frouin (24) recognized the fact that the phosphatide yielded on hydrolysis a large amount of reducing sugar which these authors regarded as glucose. Dienes and Schoenheit (25) have described phosphatide fractions that contained phosphorus and nitrogen together with

varying amounts of glucose. Koganei (26) claims, on very slight experimental evidence, that the bacillus contains phrenosin, kerasin, sphingomyelin and cephalin. It is evident, therefore, while many observations have been reported upon the presence of phosphorus in the bacillary fat which indicated the presence of phosphatide, that very little evidence has been presented, previous to our work, concerning the actual composition of the bacterial phosphatides.

Acid hydrolysis of the phosphatide. In preliminary experiments it was found that the phosphatide when hydrolyzed with 5 per cent sulfuric acid yielded about 66 per cent of fatty acids and about 33 per cent of water-soluble material. Larger amounts of the phosphatide were then hydrolyzed and attempts were made to separate the cleavage products quantitatively.

The mixed fatty acids were first separated by means of the lead soap-ether procedure of Gusserow (27)-Varrentrapp (28) into solid and liquid fatty acids. The solid fatty acids obtained from the ether-insoluble lead soap was found to be practically pure palmitic acid because after it had been recrystallized a few times from methyl alcohol and acetone it was obtained as snow-white crystals which melted at 62° and the molecular weight determined by titration was 258–259. Pure palmitic acid melts at 62–63° and its molecular weight is 256.

EXAMINATION OF THE LIQUID FATTY ACIDS: *Reduction of the unsaturated acid and the isolation of stearic acid.* The liquid fatty acids obtained from the ether-soluble lead soaps formed a brownish oil with an iodine number of 30–31. The low iodine number indicated that a large proportion of some liquid saturated fatty acid was present. Much time was consumed in finding a suitable method for the separation of unsaturated and saturated liquid fatty acids. The only feasible method that we have been able to devise for this separation consists in reducing the unsaturated acid with hydrogen and platinum oxide (29). In this process the unsaturated acid is converted into stearic acid and the latter can be removed from the mixture by repeating the lead soap-ether treatment.

The reduced acid isolated from the ether-insoluble lead salt was obtained as snow-white crystals which melted at 69–70° and its molecular weight determined by titration was 281, thus proving that the substance was stearic acid. It is evident from a consideration of the iodine number of the mixed liquid acids and the amount of stearic acid obtained after reduction that the unsaturated acid was oleic acid.

ISOLATION OF THE LIQUID SATURATED FATTY ACID: *Preparation and*

properties of phthioic acid. From the ether-soluble lead soaps, after removing the reduced acid as just described, we obtained the liquid saturated fatty acid. The new acid possesses very interesting properties. It is a saturated compound because it absorbs neither bromine nor iodine and the analytical values obtained on combustion agree with a saturated acid. At room temperature the acid is an oily liquid but it solidifies on cooling in ice water. It is soluble in all proportions in organic solvents and it is optically active. In alcoholic solution it shows $[\alpha]_D^{20} = +1.66°$. Values obtained on titration indicate that the molecular weight is high. The following values were obtained on analysis:

Found, C, 76.17, 76.22; H, 12.88, 12.84 per cent.

Found, molecular weight by titration, 309, 313.

The substance is not homogeneous but contains at least two acids that differ in optical rotation. The acid forms a crystalline salt with benzylpseudothiourea and this salt can be separated into two fractions by crystallization. The less soluble fraction yields an acid which is optically inactive while from the more soluble fraction an acid is obtained with an optical rotation twice as high as the original material.

We have found in later experiments that all of the lipoid fractions of the bacillus contain similar liquid saturated fatty acids and the acetone-soluble fat is particularly rich in these acids. As will be described later it has been possible to separate and to purify the optically active liquid fatty acids obtained from the acetone-soluble fat into more homogeneous fractions.

The interesting observation has been made in Doctor Sabin's laboratory (30) that the optically active liquid fatty acids possess a similar biological reaction to the phosphatide itself. The pure acids when injected into normal healthy animals cause the proliferation of monocytes, epithelioid cells and giant cells with the formation of tubercular tissue. For this reason the acid has received the name phthioic acid (31) from the Greek word phthio.

The water-soluble constituents. The water-soluble cleavage products which are liberated by acid hydrolysis of the phosphatide have been separated and identified. It has been found that glycerophosphoric acid, inosite (32), mannose (33), and some other reducing hexose sugar is present (34). The nature of the latter sugar has not been determined. It is levorotatory and it gives an osazone which is identical with glucosazone.

The separation of the water-soluble constituents is not very difficult

and can be accomplished in a comparatively short time (35). The glycerophosphoric acid is first removed as the barium salt by precipitation with alcohol. The mannose is next removed by means of phenylhydrazine in the form of the well crystalline mannose phenylhydrazone. The solution is then freed of the excess of phenylhydrazine by shaking with benzaldehyde. After filtering, the filtrate is concentrated to dryness when the inosite separates in crystalline form and the crystals are washed free from adhering syrup with alcohol. The remaining hexose sugar is obtained as a syrup by evaporating the filtrate and washings to dryness.

TABLE 3

Cleavage products from bacterial phosphatides

	HUMAN	AVIAN	BOVINE	TIMOTHY	LEPROSY
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Total ether soluble	66–67	55–56	57–58	60	62.2
Palmitic acid	30.5	18.4	27.0	20.0	18.6
Oleic acid after reduction to stearic acid	12.8	18.4	7.0	5.6	13.8
Liquid saturated fatty acids presumably mixtures of tuberculostearic and phthioic acids	20.9	14.1	16.0	18.0	13.5
Total fatty acids recovered	64.2	53.7	50.0	43.6	45.9
Water soluble constituents	33.34	46–47	43–44	40.0	38.0
Mannose	9.2	13.3	6.7	9.5	5.2
Inosite	8.9	3.0	3.5	2.2	0.6
Other sugars	12.3				20.6
Glycerophosphoric acid	5.4	6.0	9.9	10.0	

The methods of analysis outlined above have been applied in the study of other phosphatide preparations that have been isolated from the avian (36) and bovine tubercle bacilli (37), the timothy bacillus (38) and the leprosy bacillus (39). The results that have been obtained are shown in table 3.

The nitrogen compounds in the phosphatides. All of the phosphatides contain small amounts of nitrogen but the nature of the nitrogen compound has not been definitely established. It has been impossible to isolate either choline or any other nitrogenous base after the phosphatides have been hydrolyzed. In the case of the human phosphatide nearly all of the nitrogen can be accounted for as ammonia by distillation with dilute alkali. It remains, however, a problem of future research to determine the form in which the nitrogen is present in these compounds.

ALKALINE SAPONIFICATION OF THE PHOSPHATIDE: *Isolation of a new polysaccharide, maninositose.* (40) When the phosphatide is boiled with 1 per cent alcoholic potassium hydroxide the saponification proceeds only to the extent that the fatty acids are split off. The carbohydrate group remains apparently intact as an insoluble mass in the alcoholic solution. The material is easily soluble in water and by means of lead acetate it can be separated into a neutral and an acid fraction. The neutral substance is easily soluble in water and it is precipitated as a snow-white amorphous powder when the concentrated solution is poured into absolute alcohol. The substance gives no reduction when boiled with Fehling's solution. It is optically active and in aqueous solution it shows $[\alpha]_D^{23} = +57°$. After the substance has been hydrolyzed with 5 per cent sulfuric acid the only cleavage products that could be isolated were mannose and inosite. This substance which has received the name maninositose apparently represents a new type of polysaccharide or possibly a glucoside. It is a matter of interest to notice that inosite which is so universally distributed in plant and animal cells is here found for the first time in combination with a hexose sugar such as mannose.

Relation of the bacillary phosphatide to ordinary lecithin. The chemical composition of the bacillary phosphatides differs in many particulars from that of the usual plant or animal lecithin (41). The name phosphatide has been retained, nevertheless, to designate these compounds because they represent a class of phosphorus containing substances which are easily soluble in ether and which are precipitated by acetone. The bacillary phosphatides contain a much lower percentage of phosphorus than the ordinary phosphatides and as a rule they contain only traces of nitrogen. They differ especially from ordinary phosphatides in that they contain a large amount of a new type of polysaccharide with which the fatty acids are apparently combined.

Chemistry of the acetone-soluble fats. The acetone-soluble fats from all of the acid-fast bacteria were separated from the other lipoids by reason of their solubility in ice cold acetone. They form brown soft salve-like masses possessing peculiar perfume-like odors and containing large amounts of free fatty acids. The fats are free from phosphorus, nitrogen and sulfur. It was assumed at first that these fats were glycerides but this is apparently not the case because after saponification very little glycerol can be isolated. A water-soluble syrupy substance is obtained in place of glycerol which probably represents some higher polyhydric alcohol or carbohydrate but these compounds have not yet

been obtained in pure form. All of the acetone-soluble fats contain traces of volatile fatty acids having the odor of butyric acid but the amount found has been too small to permit of a definite identification.

The constants of the acetone-soluble fats from the human (42) and bovine tubercle bacilli (43) and the timothy bacillus (44) that have been examined in this laboratory are given in table 4.

The values obtained in the analyses of the acetone-soluble fats are not comparable with other published analyses of tubercle bacilli fat because other investigators have generally employed mixtures containing varying proportions of the total lipoids. In the work reported by Goris (4), however, an attempt had been made to separate the total lipoids into wax, phosphatide and acetone-soluble fat. The separation must have been incomplete because the acetone-soluble fat was found

TABLE 4

Constants of the acetone-soluble fats

	HUMAN	BOVINE	TIMOTHY
Melting point	33°	38°	Liquid
Iodine no.	52.6	48.4	72.3
Acid value	60.3	117.5	62.7
Saponification value	203.6	171.2	231.8
Ester value	143.3	53.7	169.1
Reichert-Meisel value	3.9	4.1	Trace
Unsaponifiable matter	10.3	9.7	22.1

to contain a large amount of unsaponifiable matter similar in properties to the so-called mycol.

The small amount of unsaponifiable matter which we obtained after saponifying the acetone-soluble fat was an unsaturated oil of agreeable perfume-like odor. It gave no sterol color reactions and no solid substance separated on cooling. It is evident therefore that substances such as mycol described by Tamura (23) and the higher alcohols mentioned by Bürger (22) must have been derived from the wax fractions.

Hammerschlag (2) believed that the bacillary fat consisted of tripalmetin and tristearin with little or no triolein. DeSchweinitz and Dorset (45) reported that the fat contained lauric, palmitic and arachidic acids while Bulloch and Macleod (46) mention lauric, myristic, isocetic and oleic acids. Baudran (19) refers only to stearic and oleic acids. Goris (4) who used large quantities of mixed human and bovine bacilli in his investigations states that the fat contained free fatty acids and

glycerides of caproic, oleic, palmitic, stearic and arachidic acids, together with certain unidentified liquid fatty acids. The presence of volatile fatty acids was first noted by DeSchweinitz and Dorset (45) and similar observations were reported by Kresling (18) and by Goris (4) who regarded the volatile acid as butyric acid.

The neutral fat is always referred to as glycerides by earlier investigators but the nature of the alcohol that is present has never been determined. The presence of glycerol in the fat as indicated by a positive acrolein test has only been reported by Bulloch and Macleod (46), by Agulhon and Frouin (24) and by Koganei (26).

Saponification of the fat: Separation of the fatty acids. After the fat had been saponified with alcoholic potassium hydroxide the unsaponifiable matter was removed by extraction with ether. The mixed fatty acids were isolated and separated into solid and liquid acids by means of the lead soap-ether procedure. The solid acids were converted into methyl esters and the latter were separated by fractional distillation in high vacuum.

The liquid fatty acids. The crude liquid fatty acids obtained from the ether-soluble lead salts were very dark-colored oils. The iodine numbers were low, thus indicating that large amounts of liquid saturated fatty acids were present. It was impossible to apply catalytic reduction directly to the crude liquid acids because some impurity was present which poisoned the catalyst. It was therefore necessary to convert the mixed acids to methyl esters and to distill the latter in a vacuum. The procedure entailed, unfortunately, a considerable loss of material but the unsaturated fatty acids could now be reduced with hydrogen and platinum oxide.

The reduced acid obtained after catalytic reduction was in every case found to be stearic acid and it was removed by repeating the lead soap-ether treatment. When we consider the amount of stearic acid which could be isolated in conjunction with the iodine number of the mixed liquid acids it becomes evident that the unsaturated fatty acids present in the acetone-soluble fat must have consisted either of linoleic acid or linolenic acid (42).

THE BIOLOGICALLY ACTIVE LIQUID SATURATED FATTY ACIDS. *Isolation of tuberculostearic acid and phthioic acid.* It has been a matter of much interest both from the chemical and biological standpoint to find that the acetone-soluble fats were particularly rich in the new biologically active liquid saturated fatty acids which were first isolated from the phosphatides. By utilizing the acetone-soluble fats as the source of

these interesting compounds it has been possible to obtain larger amounts of these acids and we have been able to purify them more thoroughly than could be done with the small quantities obtained at first.

The new acids were isolated from the ether-soluble lead salts after removing the reduced acid (47). They were converted to methyl esters and the esters were fractionated in high vacuum in an all-glass distilling apparatus when two principal fractions were obtained. The first fraction was a faintly yellowish oil, boiling point 140–145° at 0.0005 mm. pressure while the second fraction was a thick yellowish oil, boiling point 200° at 0.001 mm. pressure.

The low boiling ester yields on saponification a fatty acid which is a nearly colorless oil at room temperature. On cooling it forms a white solid mass which liquefies at 14–15°. The substance is an isomer of stearic acid, $C_{18}H_{36}O_2$, that has received the name tuberculostearic acid. It is optically inactive and according to investigations in Doctor Sabin's laboratory (3) it possesses no cell stimulating properties.

The high boiling fraction of the ester is of more immediate interest because it contains the optically and biologically active material. On saponification the ester yields a saturated fatty acid corresponding in composition and molecular weight to a hexacosanic acid, $C_{26}H_{52}O_2$. At ordinary temperature it is a solid that melts at 28°. In alcoholic solution it shows a specific dextrorotation of about 8°. In order to indicate the relation of this acid to tuberculosis it has been named phthioic acid.

During the past year we have attempted to carry out further purifications of phthioic acid. By the slow and careful fractionation of the methyl ester in a very high vacuum it was possible to separate it into dextro- and levorotatory fractions. The purest samples obtained showed $[\alpha]_D^{20} = +10.5°$ and $[\alpha]_D^{20} = -5.0°$ respectively (48). There is but slight difference in chemical composition between these fractions but they show distinct differences in biological behavior. The cell stimulating property is associated only with the dextrorotatory acid (49).

All of the lipoid fractions from the human tubercle bacillus contain optically active liquid saturated fatty acids and the assumption seems justified that they represent mixtures of tuberculostearic acid and phthioic acid. Analogous liquid saturated fatty acids have been found to occur in all of the lipoid fractions from the avian and bovine tubercle bacilli, the timothy bacillus and the leprosy bacillus that have been examined. The acids from these bacteria exhibit similar biological reactions to phthioic acid (50) but they differ to the extent that they are optically

inactive. Unfortunately lack of time and material has prevented complete chemical investigations of these interesting compounds.

The composition of the acetone soluble fats that have been analyzed is shown in table 5.

TABLE 5

Percentage composition of the acetone-soluble fats

	HUMAN	BOVINE	TIMOTHY
	per cent	*per cent*	*per cent*
Water-soluble compounds	6.6	9.3	4.8
Fatty acids	83.3	83.9	74.0
Unsaponifiable matter	10.3	13.1	22.1
Solid fatty acids	30.3	32.4	15.9
Liquid fatty acids	50.6	45.0	44.6
Unsaturated fatty acids after reduction to stearic acid	12.5	10.6	12.0
Saturated liquid fatty acids	38.0	18.6	19.2

TABLE 6

Fatty acids contained in the acetone-soluble fats

	HUMAN	BOVINE	TIMOTHY
Butyric acid $C_4H_8O_2$	Trace	Trace	Trace
Palmitic acid $C_{16}H_{32}O_2$	Large amount	Large amount	Large amount
Stearic acid $C_{18}H_{36}O_2$	Small amount	None found	None found
Cerotic acid $C_{26}H_{52}O_2$	Trace	Small amount	None found
Linoleic acid $C_{18}H_{32}O_2$	Small amount	Small amount	Small amount
Linolenic acid $C_{18}H_{30}O_2$			
Tuberculostearic acid $C_{18}H_{36}O_2$	Large amount	Large amount	Large amount
Phthioic acid $C_{26}H_{52}O_2$			

Except for traces of butyric acid all of the fatty acids that could be found in the acetone-soluble fats belong to the C_{16}, C_{18} and C_{26} series as shown in table 6.

It is very probable that other acids analogous to phthioic acid will be discovered when larger quantities of material are available for investigation. We have already observed some evidence of small amounts of higher acids in this fraction which for lack of material could not be purified.

Mixed lipoids from the human and bovine tubercle bacilli. It was a

matter of interest in this comparative investigation to examine a specimen of lipoids obtained from a mixture of human and bovine tubercle bacilli. The fat had been obtained by extracting the dried bacteria with toluene and was therefore a composite sample of the total lipoids. The solvent had been removed by distillation and the fat had been kept in a closed container for about 7 years (51). This material was kindly placed at our disposal by Prof. Treat B. Johnson.

No attempt was made to separate this fat into fractions. It was saponified and the cleavage products were determined with the special object in view of isolating any liquid saturated fatty acids that might be present (52). A comparatively large amount of liquid saturated fatty acids was obtained and they were separated by distillation of the methyl esters into fractions which on saponification yielded optically inactive tuberculostearic acid and optically active phthioic acid.

The unsaturated fatty acids after reduction with hydrogen and platinum oxide were found to consist of a large amount of stearic acid and a small amount of a hexacosanic acid which melted at 82–83° (53).

Incidentally it was found that the fat contained a large amount of a polysaccharide which gave reducing sugars on acid hydrolysis (54). From the hydrolysis mixture it was possible to isolate mannose, inosite, galactose and d-arabinose. It seems evident therefore that the substance was a mixture, as would be expected in this case, of the polysaccharides which occur in the phosphatide and in the so-called purified wax.

Chemistry of the wax fraction of the human tubercle bacillus. All of the acid-fast bacteria contain relatively large amounts of wax-like material but so far we have only had time to determine the composition of the wax fractions of the human tubercle bacillus (55). The so-called wax represents 46 per cent of the total lipins and about 11 per cent of the dried bacteria. The crude wax obtained by extraction with chloroform is a yellowish brittle solid which melts at 50–51°. For purification the material was precipitated several times from ethereal solution with acetone or methyl alcohol when a nearly white amorphous powder was obtained. The "purified wax," representing nearly 80 per cent of the original material, melts with decomposition at 200–205° and it contained 0.4 per cent of phosphorus and 0.7 per cent of nitrogen. The mother liquors yielded on evaporation of the solvent a yellowish salve-like mass which was designated "soft wax" and it was practically free from phosphorus and nitrogen.

Composition of the purified wax. The purified wax is very stable.

It is hydrolyzed with difficulty and apparently no change takes place when it is refluxed with dilute aqueous acid. When the substance is boiled with alcohol containing 1 per cent of hydrochloric acid it yields about 70 per cent of ether soluble and some 40 per cent of water soluble constituents. The latter contain reducing sugars consisting of pentoses and hexoses. When the purified wax is boiled with alcoholic potassium hydroxide it is gradually saponified with the formation of an oil which is insoluble in alcohol and a sticky mass insoluble in organic solvents but which is easily soluble in water. The water-soluble material obtained in this case represents a stable polysaccharide which after hydrolysis with dilute sulfuric acid yields pentose and hexose sugars.

The ether-soluble material obtained on saponification contains a small amount of fatty acids. The fatty acids were separated into solid and liquid acids by the lead soap-ether treatment. The solid acids appeared to consist of a eutectic mixture of palmitic and stearic acids. The liquid acids had a very low iodine number and after catalytic reduction it was possible to isolate a small amount of stearic acid. The main portion of the fatty acids was a mixture of liquid saturated acids that differed from the crude phthioic acid as obtained from the other lipoid fractions in that it was levorotatory. The specific levorotation was $-1.65°$. The principal ether-soluble constituent was a very stable wax-like substance that we have described under the name unsaponifiable wax. This interesting compound will be referred to more fully in a subsequent paragraph.

The polysaccharide fraction is only partially hydrolyzed when boiled with alcohol and dilute acids. For complete hydrolysis, as determined by the maximum reduction with Fehling's solution, the substance must be refluxed for several hours with dilute sulfuric acid. The cleavage products which are formed have not been determined completely but we have isolated and identified the following sugars, d-arabinose, mannose and galactose, together with traces of glucosamine and inosite (56). It was a matter of particular interest to find that this unique polysaccharide yielded over 35 per cent of d-arabinose, a pentose which is so rarely found in nature. The extraordinary stability of the polysaccharide is indicated by the fact that we have isolated it after the wax had been refluxed for 8 hours with 10 per cent alcoholic potassium hydroxide and the substance still showed a specific precipitin reaction with immune serum in dilutions as high as 1:3,000,000 (57).[1]

[1] We are indebted to Dr. Michael Heidelberger of Columbia University for determining the precipitin reaction.

The phosphorus contained in the purified wax is found among the water-soluble constituents and mainly in organic combination. A small quantity of a water-soluble barium salt of an organic phosphoric acid was isolated but the substance contained nitrogen and could not be identified.

It is evident from the composition of the so-called purified wax and from the cleavage products that are obtained on saponification that the substance is not a wax in the ordinary meaning of that term but that it represents a complex phosphatide containing a large proportion of a polysaccharide combined in its molecule.

Earlier analyses of the tubercle bacilli wax have been reported by Goris (4) and by Lewkowitsch (58) but the only cleavage products described are fatty acids and a higher alcohol, no mention being made of the polysaccharide constituents.

Composition of the soft wax. The composition of the fraction designated soft wax was found to correspond to that of a complex glyceride (59). The substance gave on saponification 95 per cent of ether soluble and 5 per cent of water-soluble constituents. The water-soluble component was obtained as a syrup which gave a positive acrolein reaction and presumably it consisted of glycerol. No polysaccharide or reducing sugars could be detected.

The ether soluble material contained 13 per cent of a neutral perfume-like substance, 12 per cent of unsaponifiable wax and 69 per cent of mixed fatty acids. The fatty acids were separated into (*a*), solid saturated fatty acids, m.p. 56–57°, presumably a mixture of palmitic and stearic acid; (*b*), liquid fatty acids containing a small amount of unsaturated fatty acids and a large amount of liquid saturated fatty acids analogous to crude phthioic acid, m.p. 22.5° and $[\alpha]_D^{23} = +4.85°$.

The unsaturated fatty acids on catalytic reduction were converted mainly to stearic acid, m.p. 67–68° but a small amount of a higher fatty acid was also present. The acid melted at 79–80° but the amount obtained was too small to permit of adequate purification. We believe, however, that the acid is identical with the similar reduced acid which was isolated from the mixed lipoids from the human and bovine bacilli and which was identified as a hexacosanic acid, $C_{26}H_{52}O_2$. The occurrence of an unsaturated hexacosanic acid in the bacterial lipoids is a matter of interest because unsaturated fatty acids of such high molecular weight have not previously been reported.

The unsaponifiable wax. The first reference to the tubercle bacilli

wax was made by Aronson (60) and Kresling (18) reported that the unsaponifiable matter consisted of some higher alcohol. Similar observations have been reported by Bulloch and Macleod (46), Dorset and Emery (61), Auclair and Paris (20), Fontes (62), Panzer (63) and by Kosniewski (64). The chemical composition and properties of this higher alcohol were studied by Tamura (23) who described it as a monohydric alcohol of the formula $C_{29}H_{56}O$ to which the name mycol was given. Bürger (22) described other higher alcohols which he had isolated from the bacillary fat. Goris (4) in his work refers to the presence of mycol but apparently he did not analyze the substance.

The unsaponifiable wax (65) which we have described must correspond to the higher alcohols observed by former investigators because it is the only cleavage product obtained from the lipoid fractions that possessed the properties of a higher alcohol. In several particulars, however, the properties of the unsaponifiable wax differed from those reported for mycol and other higher alcohols.

The unsaponifiable wax is an extremely stable compound and it can be refluxed for long periods of time with alcoholic potassium hydroxide, sodium ethoxide or with acidified alcohol without showing any changes either in properties or in composition. The substance is precipitated as a snow-white amorphous powder either by cooling the ethereal solution or by adding acetone but it has not been obtained in crystalline form. It is a saturated compound which melts at 56–57° and it appears to contain one carboxyl and two hydroxyl groups because it forms salts and also yields acetyl derivatives. When the substance is boiled with hydriodic acid in the Zeisel apparatus a small amount of a volatile iodide is formed which if calculated as isopropyl iodide corresponds to 4 per cent of glycerol.

When the unsaponifiable wax is heated to a temperature of 250–300° either at a pressure of about 20 mm. or in a high vacuum it cracks and a crystalline fatty acid distills over leaving a nearly colorless non-volatile unsaturated residue in the flask. The crystalline fatty acid obtained by distillation has been identified as normal hexacosanic acid, $C_{26}H_{52}O_2$ (66), which melts at 88–89° and it is identical with the synthetic normal hexacosanic acid prepared by Levene and Taylor (67).

The property of acid-fastness of the acid-fast bacteria is usually attributed to the fatty or waxy material contained in the bacilli. An excellent review of this subject is given by Long (6). Aronson (60) who first isolated the wax from the tubercle bacillus showed that it was acid-fast and later a similar observation was reported by Bulloch and Mac-

leod (46) while Tamura (23) found the substance called mycol was acid-fast. The various fat and wax fractions isolated in our work have been tested for acid-fastness and the unsaponifiable wax is the only fraction which possesses this property.[2]

The biological reactions of the unsaponifiable wax have been studied in Doctor Sabin's laboratory (3) and the results obtained indicate that it causes a marked increase in connective tissue cells without showing any specific stimulating effect on monocytes and epithelioid cells.

The great stability of the unsaponifiable wax, its low melting point and the formation of a volatile iodide when heated with hydriodic acid suggest that it may contain an ether linkage in its molecule such as was found in batyl and selachyl alcohols by Heilbron and Owens (68). The composition of the substance as determined by combustion and by analyses of the potassium and silver salts and of the acetyl derivative agrees with the unusual formula $C_{94}H_{188}O_4$. While we attach no particular importance to a formula calculated from analysis of an amorphous compound yet all the determinations that have been made indicate that the substance has a high molecular weight.

Are sterols present in acid-fast bacteria? Sterols of some kind are always associated with the fats obtained from all higher plants and animals as well as from yeasts. In the examinations of the fats and lipoids from the acid-fast bacteria we have not been able to isolate even traces of true sterols or any substance which gives the usual sterol color reactions. It is not impossible that sterols may be present in the bacilli in some chemical combination which is insoluble in the solvents that we have employed for the extraction of the bacterial fats. The matter has not been exhaustively investigated and we can only state that up to the present time we have not found any sterols in the materials that we have examined. In this respect our results agree with those reported by Hammerschlag (2), Aronson (60), Kresling (18), Agulhon and Frouin (24), Panzer (63) and Goris (4).

SUMMARY

The work described in this review represents an attempt to separate the lipoids of the tubercle bacilli and of a series of related pathogenic and non-pathogenic acid-fast bacteria by systematic fraction-

[2] We are indebted to Dr. W. L. Kulp, Department of Bacteriology, Yale University and to Dr. M. C. Kahn, Cornell University Medical College for testing the lipoid fractions for acid-fastness.

ation into special groups such as phosphatide, acetone-soluble fat, and wax.

The chemical composition and biological reactions of the various fractions have been studied and the results of this work indicate that the bacillary lipoids occupy a unique position. Chemically they differ decidedly in composition from the usual plant and animal lipins and they possess interesting biological properties.

One is forced to conclude from the consistent results that we have obtained in the analyses of all of the lipoid fractions that the acid-fast bacteria elaborate a series of new higher fatty acids. These acids are either liquid at ordinary temperature or low melting solids and the higher members are optically active. Whether the optical activity depends upon a ring structure similar to that of the Chaulmoogric series cannot be determined from the data now available and it remains a problem for future research to determine the chemical constitution of these interesting compounds. The constant recurrence of isomeric hexacosanic acids in all the fractions is noteworthy. At least one unsaturated hexacosanic acid is present and several isomers of solid saturated hexacosanic acids have been found including the normal straight chain acid. The optically active phthioic acid belongs to this group.

Phthioic acid possesses specific biological properties but it cannot be stated definitely at this time that the biological activity is due to the acid itself since it may depend upon traces of other substances associated with this acid fraction. However, the activity remains after numerous fractionations in high vacuum of the methyl ester.

The fat fraction which is soluble in cold acetone represents a mixture of free fatty acids and neutral fat. The neutral fat is not a true glyceride because it yields only a very small amount of glycerol on saponification. It is apparently an ester of fatty acids with some higher polyhydric alcohol but the latter substance has not yet been identified.

The phosphatides and the so-called purified wax contain large amounts of polysaccharides which differ not only in biological reactions but also in their chemical composition. The polysaccharide contained in the phosphatide gives no precipitin reaction with immune serum and on hydrolysis it yields inosite, mannose and some other sugar which has not yet been identified. The polysaccharide isolated from the wax gives a precipitin reaction with immune serum and on hydrolysis it yields mainly d-arabinose, mannose and galactose together with some other unidentified sugars.

The principal constituent of the wax fraction is a very stable, snow-

white, amorphous substance which provisionally has been designated unsaponifiable wax. The substance possesses the properties of a higher hydroxy acid and it is acid-fast.

The lipoid fractions apparently contain no sterols.

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The chemical studies on the lipoids of the acid-fast bacteria form a part of a systematic chemical and biological coöperative investigation on tuberculosis which is conducted under the auspices of the Medical Research Committee of the National Tuberculosis Association. We desire to express our thanks to this Committee for financial assistance, and especially to its Chairman, Dr. William Charles White, to whose efforts the success of this coöperative research is largely due.

The biological experiments carried out in Doctor Sabin's laboratory of the Rockefeller Institute have been of very great value in guiding the chemical work towards the biologically active fractions and our thanks are due to Dr. F. R. Sabin and Dr. C. A. Doan.

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LOCAL ANESTHETICS

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HISTORICAL. "Up to 1884," wrote Carl Koller, "the only method of local anesthesia known and not very frequently practiced was the Richardson ether spray which acted by freezing and which was used for operations on subcutaneous abscesses and for similar operations."

It is true that Gaedecke had isolated in 1855 an alkaloid "erythroxyline" from Erythroxylon coca which was undoubtedly identical with cocaine. Wöhler (1860) had noticed that the alkaloid cocaine (probably identical with Gaedecke's erythroxyline) which his pupil Niemann had just isolated from the leaves of the Peruvian shrub Erythroxylon Coca "has a bitter taste and has the peculiar effect upon the nerves of the tongue of making the point of contact essentially numb, and almost devoid of sensation." B. von Anrep in 1879 studied the pharmacological effects of cocaine and found that when it was injected subcutaneously the area of skin above the injection became insensitive to the prick of a pin, and he actually recommended that it might be used clinically as a local anesthetic. However, he did not follow up this suggestion.

H. Braun states that in 1870 French laryngologists, Fauvel and Saglia, were treating painful affections of the larynx and pharynx with extracts of coca leaves and that Coupard and Borderau in 1880 showed in animals that solutions of cocaine applied to the conjuntivae abolished the lid reflex.

However, the real introduction of local anesthesia into the practice of medicine and surgery dates from 1884, when Karl Koller[1] and Sigmund Freud, two young Vienna physicians, made a systematic study of various alkaloids in the hope of finding a substitute for morphine. They were most anxious to obtain this in order to furnish relief for the pains which one of their friends, an eminent young physiologist, was then suffering caused by a painful lesion on his thumb. In the course of these experiments, Koller accidentally dropped a little cocaine solu-

[1] For many years Doctor Koller has been in active practice in ophthalmology in New York City.

tion into his eye, noted the anesthesia that resulted, demonstrated that conjunctival and corneal anesthesia result from application of cocaine to the eyes of guinea pigs, rabbits and dogs, and then applied the drug clinically in ophthalmological practice and ophthalmological surgery. He recommended it also to his colleague, Jellinek, who used it in laryngological surgery; and after this the use of cocaine for infiltration anesthesia. Koller reported his results to the Ophthalmological Congress at Heidelberg in September 1884, and its application to surgery rapidly became general. Within six weeks after his report it was being used in America, and the most active of its advocates, William Stewart Halsted (later Professor of Surgery at the Johns Hopkins University) was able to report within less than a year, successful local anesthesia in over a thousand surgical cases.

Within two months after Koller's discovery (before Nov. 26, 1884) Halsted[2] and his assistant Hall investigated the effect of injecting cocaine solution into the vicinity of their own nerves and found that "injecting subcutaneously six minims (of a 4 per cent solution) on the dorsal surface of the forearm caused complete loss of sensation over an area extending *downward* as far as the lower end of the ulna, from three quarters of an inch to an inch wide above, and half an inch wide below, obviously following the distribution of a cutaneous branch of the ulnar nerve. There was no diminution of sensibility above the point at which the needle was introduced." They demonstrated this nerve block on other nerves, and on November 26, 1884, Halsted introduced nerve block into practical dentistry by injecting cocaine solution into the vicinity of Doctor Hall's infraorbital foramen in order to anesthetize his sensitive dentine for a dental operation (Hall, 1884). Halsted in 1885 also experimented successfully with dilute cocaine solutions aided by infiltration and local tissue tension and thus antedated Schleich in the use of very dilute solutions in anesthesia.

In 1885, Corning, also an American, demonstrated the successful production of spinal anesthesia in a dog, without ill effects to the animal,

[2] With his characteristic diffidence Halsted never wrote an adequate publication of this work. He refers to it in a very brief article on the local anesthetic effects of water and of cocaine in dilute solutions, but his priority is clearly put on record by his first assistant, R. J. Hall (1884), and by a dentist, Raymond (1885), both of whom mention operations performed on patients whose nerves were blocked by the injection of cocaine into the nerve, and state that these injections were made by Doctor Halsted. Halsted's priority in this field is proclaimed by Matas and has been awarded recognition by the National Dental Association.

but this method was not applied in general surgery until 1900 when August Bier demonstrated its practicability. The safer substitutes for cocaine, as well as the control of their diffusion by the use of mixtures whose specific gravity is different from that of the spinal fluid, introduced by E. Erhardt (1912) and perfected by G. Pitkin (1928), have brought this method into very general use.

Next in importance to Koller's introduction of cocaine must rank the demonstration by Einhorn (1899) that esters of aminobenzoic acid have local anesthetic properties when brought into contact with nerves or nerve endings and that their water soluble basic derivatives are effectual substitutes for cocaine. His synthesis of novocaine (procaine) as a much less toxic local anesthetic in 1905 may truly be said to have introduced the modern era in local anesthesia.

The two other most important contributions upon which the present status of local anesthesia is based may be said to be H. Braun's demonstration (1903) that the addition of epinephrin to local anesthetics greatly prolongs and intensifies their action, and A. L. Tatum's experimental proof that the administration of soporific drugs before the anesthetics greatly diminishes their toxicity.

General properties of local anesthetic action. A. *Physiological.* None of the usual local anesthetics anesthetize the intact human skin, though Macht (1916) has shown that after prolonged immersion in a solution of cocaine the intact skin may absorb small amounts of the drug.

When the skin is scarified, however, and the nerve endings are thus exposed, conditions are altered and the local application of most of the local anesthetics as well as the insoluble esters of p-aminobenzoic acid (ethylaminobenzoate, orthoform, butescine, etc.) becomes effective. This is also true in the case of eroded or ulcerated mucous membranes in the mouth, stomach, etc.; so that ethylaminobenzoate, orthoform, etc. are found to deaden the pain due to gastric ulcers.

All the commonly used local anesthetic substances act much as does cocaine in depressing the sensitivity of the nerves to pain, heat and cold.

Goldscheider (1886) showed that on injection of cocaine, sensitiveness to tickling first disappears, then heat and cold, then pain, while touch and pressure were less affected. When the action passes off the sensations return in the reverse order. He found that during recovery from anesthesia a hyperesthetic state may exist in which application of water at 39° to the cocainized area of skin may cause a sensation of

intense pain, but not of heat. He claimed that cocaine usually raises the threshold of sensation to touch more than to pain and less than to heat and cold. On the conjunctiva, on the other hand, pain was affected more than touch. This is the more usual order of sensory depression.

Rohde (1921) in the main confirmed Goldscheider's early observations but found that in local anesthesia by intradermal injection there was complete loss of temperature and pain sensation, but only partial loss of light touch, and still less diminution of deep pressure sensation. During subsidence of the anesthesia, pressure, touch, pain and lastly temperature sensation return in the order named. However, most observers find that light touch (tested by a very thin wisp of cotton wool) is one of the first sensations to disappear and returns after return of pain sensation.

After subsidence of the anesthesia a certain amount of hyperesthesia may supervene, appearing first toward the periphery of the anesthetized area and gradually passing inwards. The intensity of this hyperesthesia varies with different anesthetics.

On the intact cornea von Frey (1929) finds that pain sensation becomes anesthetized by the application of cocaine base a little earlier than sensations to heat and cold presumably because the former end in bare nerve fibrils near the surface while the latter fibers end in the more complex end corpuscles, which are less easily penetrated by the anesthetic.

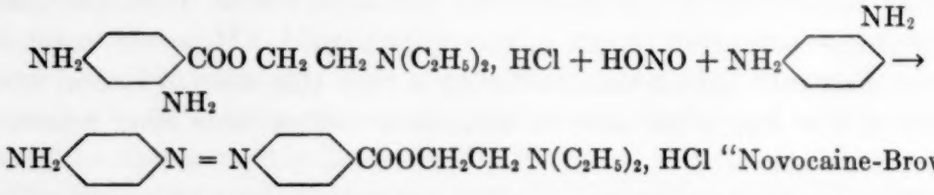
On the mucous membrane of the mouth and tongue there is great variation in the action of the various local anesthetics. Two per cent cocaine and two per cent butyn applied to the intact mucous membrane anesthetize the tongue to pain and to bitter taste, but not to sweet, sour or salt tastes, nor to heat, cold, or pressure. Tutocaine (2 per cent) sometimes has the same effect, but frequently fails to do so. Procaine (4 per cent) is practically without effect (Hirschfelder, 1931).

The sensory fibers of a mixed nerve and especially the nerve endings are depressed more than the motor fibers. This fact was strikingly illustrated by Lipschitz and Weingarten (1928) and Laubender (1928). They found that the application of methylene blue, which as Ehrlich (1886) had shown can selectively stain the sensory nerve endings, increases the anesthetic action of cocaine on the frog's skin.

The order of intensity of anesthesia to touch, upon intradermal injection in man, is given by Hoffmann and Kochmann (1914) as cocaine > alypine > eucaine > ethylhydrocupreine (optochin) > psicaine > tropacocaine > quinine > urea > procaine > saligenin. Cocaine, psicaine,

tropacocaine, tutocaine and procaine usually inhibit afferent impulses in about half the concentration that paralyzes the efferent, but stovaine has the opposite effect, and that of alypine is about equal on both sets of fibers (Sollmann, 1917–18; Boehminghaus and Kochmann, 1928).

Fulton (1921) was able to convert procaine (novocaine) into a vital stain (novocaine-Brown) by diazotizing it with M-phenylendiamine both in vitro and in vivo.



He found that this dye had the same local anesthetic action as procaine, and that when applied to a frog's nerve muscle preparation it stained selectively the nuclei of the cells at the myoneural junction, indicating a curare-like action at this site. It did not, however, stain the nerve fibers, even though the substance produced local nerve block, like procaine itself.

1. Parallelism between effects of local anesthetics and general anesthetics. Schroff (1862) and R. Boehm (1862) called attention to the parallelism between the action of cocaine and that of the general anesthetics. This was further emphasized by Boehm (1901) and by the latter's pupil, O. Gros (1910). Gros showed that not only was the motion of paramecia stopped and red blood corpuscles laked by very dilute solutions of cocaine, eucaine, alypine, stovaine and procaine but on the other hand that the general anesthetics and soporifics (e.g., chloroform, amylene hydrate, ethylurethane, paraldehyde, ethyl propionate, chloralhydrate, hypnone, phenylurethane) when injected intracutaneously in man produce local anesthesia in the center of the wheal and hyperesthesia along the margin. The order of intensity of this action as well as in that of depressing efferent motor impulses when applied to the frog's sciatic nerve is the same as the order of their intensity in narcosis of tadpoles; and in general the concentration required to suppress these functions of the peripheral nerves is about six times greater than that necessary to affect the central nervous system.

Gross believes that the local anesthetics do not act as general anesthetics merely because depression of the respiratory system, etc., sets in before depression of the cerebrum. On the other hand, Gros (1929)

found that most of the hypnotics, narcotics and antipyretics also depress the peripheral nerves when directly applied to them but not as strongly as do the local anesthetics.

Tashiro (1913–14), Riggs (1919) and Niwa (1918–1919) have shown that CO_2 production in nerve fibers runs parallel to the conduction of nerve impulse, and is depressed by ether, cocaine and KCl when the nerve impulse is depressed. Tashiro (1922) and Gerard and Meyerhof (1927) have also shown that nerve activity is accompanied by a giving off of NH_3 from the fiber, and that this also is depressed by anesthetics, at least by urethane.

One striking discrepancy between the effects of substances upon local and upon general anesthetics is encountered in the actions of potassium and magnesium salts. The addition of potassium sulphate increases the potency of local anesthetics (vide infra), while magnesium sulphate, though itself a general anesthetic and a synergist of morphine, neocinchopen and other analgesic drugs, does not have this effect on local anesthetics. It is conceivable that the potassium salts merely increase the penetration of the more potent local anesthetics, while the magnesium salts delay it.

Borruttau (1901) found that alcohol, ether, chloroform and cocaine applied to the course of a nerve produced definite retardation of conduction of the nerve impulse. This fact has been confirmed by Forbes and his collaborators, Davis, Brunswick, Hopkins, McIntosh and Sefton (1926), who also found by carefully recording the action current with the string galvanometer that there is a parallelism between the magnitude of the electrical response and the velocity of its conduction along the nerve. Forbes also showed that when a nerve is exposed to the anesthetizing action of ether vapor there is a progressive diminution of the action current, and that the motor impulse to muscles disappears just when the action current ceases to be demonstrable. Since the threshold of pain sensation in centripetal impulses is probably a little higher and since the perception of pain is probably inhibited somewhat in its passage through the relaying centers in the thalamus, it does not follow necessarily that the afferent action current must be as completely diminished as the efferent current to the muscle before pain sensation is entirely abolished.

Keith Lucas and Adrian (1917) believed that in the diminution of sensation under the influence of anesthetizing substances (ether, chloroform, cocaine, etc. applied along the course of an exposed nerve) the intensity of anesthesia increased gradually and progressively, centimeter

by centimeter along the whole course of the exposed area; but Kato (1924) in Japan proved conclusively that the intensity of anesthesia produced by a given concentration of anesthetic (ether, chloroform, cocaine) is the same for a short stretch of nerve as for a long one; and quite independently, Forbes and his collaborators (1925) demonstrated that the action current obtained at different distances along such a blocked or partially blocked nerve was the same at all points, regardless of the length of nerve submitted to its action. It would appear, therefore, that when a nerve is anesthetized all the fibers are blocked about equally and at about the same level, and above this, sensation and the action current of the afferent nerve, or below it, the action current of the motor nerve is depressed uniformly.

These observed facts accord quite well with the accepted views of the physical nature of the nerve process. If we accept Cremer's hollow core theory (Kernleiter-Theorie) that the nerve fiber may be regarded as a cored conductor the center of the axis cylinder being surrounded by a more or less insulating layer of lipoid, the facts of nerve action and conduction bear a close resemblance to the electrical changes which Ralph Lillie has observed in a bit of iron wire immersed in nitric acid. The surface of the lipoid membrane would be charged negatively to the aqueous interior of the fiber; and any injury to this layer, increasing its permeability to ions would give rise to the continuous current known as the demarcation current or current of injury. The passage of the action current along the fiber, analogous to that of the current along Lillie's iron wires, would be accompanied by an increase in permeability of the lipoid surface layer of the fiber.

Loeb and Beutner showed that potassium salts which in themselves possess some local anesthetic action, diminish differences of potential (muscle, brain, and frog's skin) much more than do sodium salts.[3] Quite recently, Beutner (1931) has found that when narcotics are added to a physical system consisting of an aqueous layer floating above a non-aqueous fluid a marked negativity occurs which he regards as similar to a condition of catelectrotonus. He believes that this condition inhibits further the negativization necessary for the passage of a nerve impulse and that this explains their blocking action.

Moreover, MacDonald (1906) by the use of MacCallum's histochemical methods has shown an accumulation of potassium salts at the site

[3] For a complete discussion of this subject c.f. Lillie (1923), Hirschfelder and Serles (1926), Hirschfelder (1929).

of injury of the surfaces of nerves and finds that the exposure of nerves to anesthetic concentrations of ether and chloroform also causes an accumulation of potassium salts in the surface of the areas anesthetized.

In general, substances which increase the permeability of the lipoid layer (in concentrations less than those which destroy it) might be expected to increase nerve action and sensations, while those which decrease the permeability to water and ions might be expected to act as anesthetics.

Whether we regard the surface layer of the nerve as composed of a continuous layer of lipoid (Quincke (1888), MacDougall (1921), and McClendon (1914–26)), or of an emulsion forming a sort of mosaic with narrow channels of a protein containing water phase interposed between droplets of a fat and lipoid phase (Clowes, 1916) there is ample evidence that all the substances which cause anesthesia, narcosis and analgesia tend to decrease the permeability of this surface layer. This action is probably due to a dehydrating action upon the surface layer, or perhaps to increasing the water in oil portion of the surface layer at the expense of the more permeable channels of the water phase or oil in water phase (Clowes (1916) and Hirschfelder and Serles (1926)). It is certainly accompanied by decreased electrical conductivity (Osterhout, 1912–1928), by decreased permeability to salts (McClendon), and by decrease in the difference of the action current.

On the other hand, Bancroft and Richter (1931), who have given a good review of the physico-chemical factors involved in anesthesia, claim that the addition of narcotics and anesthetics reversibly affect the stability of colloidal suspensions of both proteins and lipoids as observed under the ultramicroscope, and they regard this effect upon the proteins as of significance equal to that of the effects upon the lipoids. They base their conclusions largely upon the experiments of Marinesco (1912) who studied under the ultramicroscope the effects of anesthetics upon the isolated spinal cord cells of the dog, and found that they seemed to render the surfaces of these cells more opaque, which he interpreted as a coagulation phenomenon. Marinesco's work, however interesting, was fragmentary and was done under conditions far removed from those of normal life. They, therefore, cannot be regarded as furnishing conclusive evidences.

B. *Physico-chemical properties of local anesthetics. 1. Effect of pH on the activity of local anesthetics.* Since all the important local anesthetics are the salts of basic substances (primary, secondary or tertiary amines)

the free base is liberated in the presence of alkali, according to the general reaction.

$$\underset{\text{(Anesthetic Salt)}}{R\equiv N,\ HCl} + NaOH \rightarrow \underset{\text{(Base)}}{R\equiv N} + NaCl + H_2O$$

Overton has shown that in general the free bases of alkaloids are much more active than their salts, probably because the salts are much more soluble in water than the free bases, and the free bases much more soluble in fats, lipoids, and organic solvents.

Bignon (1892) seems to have been the first to notice that alkalinization of cocaine increases its potency, and he introduced into surgical practice a milky alkalinized suspension of cocaine ("cocaine milk"). The activity of this preparation, however, for reasons discussed below, did not seem to be appreciably greater than that of the cocaine hydrochloride.

Gros (1910) made a systematic study of the entire subject. He found the anesthetic bases soluble in the following amounts of water: Cocaine 1 gram in 704 cc.; procaine (novocaine) 1 in 200 cc.; alypine, eucaine and stovaine much less soluble. All of these bases are very soluble in oil, cocaine least of all but nevertheless more than ten times as soluble as in water. The bases thus conform to the law of Overton and Meyer.

In this connection it is interesting that Vles and Ruppol (1920) found that the intensity of the two spectral absorption bands of cocaine (A at λ 2330 A°U and B at λ 2700 A°U) change in intensity as the pH of the solution is altered, increasing between pH 4 and pH 6, diminishing between pH 6 and 7.5 and increasing again from pH 7.5 to 10. In other words, the absorption bands are least intense in the range where anesthetic action is greatest, and vice versa.

On adding 8 cc. N/100 NaOH to 10 cc. of N/100 solutions of the hydrochlorides of cocaine, procaine, eucaine, alypine and stovaine (equivalent to 80 per cent hydrolysis), Gros found in every case that motor block set in much sooner than in the hydrochloride alone, that it lasted much longer and that on further dilution, the concentration which just gave a discernible effect (threshold concentration) was much lower than with the hydrochlorides alone. In these experiments he found the bases from four to eight times as potent as the hydrochlorides.

In intradermal wheals in man, Gros found that 0.017 per cent procaine base was the lowest concentration that produced noticeable anesthesia, corresponding to a threshold value of 0.1 per cent for the hydrochloride;

in other words, that the base is six times as potent as the hydrochloride. He found the following threshold concentrations for intradermal anesthesia in man:

$$\text{Cocaine } \frac{M}{3200} \text{ to } \frac{M}{6200}; \text{ procaine (novocaine) } \frac{M}{1600};$$

$$\text{Eucaine } \frac{M}{3200} \text{ to } \frac{M}{6400}; \text{ alypine } \frac{M}{6400}; \text{ stovaine } \frac{M}{1600}$$

The sensory nerves are thus much more sensitive to the local anesthetics than are the motor fibers; but the ratio of sensory to motor effect is not the same for all the local anesthetics;—e.g., procaine 2:1; eucaine and cocaine 4:1; alypine 8:1.

Gros calls particular attention to the fact that in order to obtain maximal effects the solution must not be so alkaline that the base is precipitated (i.e., it must be in true solution or in colloidal solution, and must never give more than an opalescence). In this state there is a large area of free surface and the positively charged ions or micellae of the anesthetic base are strongly adsorbed by the negatively charged nerve fibers or nerve endings.

If the base is aggregated into particles sufficiently coarse to give a flocculation or a precipitate the amount of free surface which is active is diminished and the effectiveness correspondingly diminishes. It was on this account that Bignon's "Cocain milk" was found to be no more effective than the hydrochloride. Gros calls attention to the fact that procaine bicarbonate made by mixing equal parts of procaine and sodium bicarbonate remains quite soluble and can even be boiled for a short time, or for a longer time in the presence of CO_2; while cocaine bicarbonate readily precipitates and thus loses its activity.

Gros' observations were confirmed by Sollmann (1918) who also found that the alkalinized solutions were more effective for surface anesthesia or upon intracutaneous use than when injected into tissues for infiltration. In the latter case unless large amounts of solution are used the pH of the injected drug quickly comes into equilibrium with that of the tissue juices.

Kochmann and Lyding (1929) found that solutions of cocaine, stovaine and alypine at pH 7.0 had the same potency as the unbuffered solutions. This merely means that at pH 7.0 very little hydrolysis takes place. In non-alkaline solutions they obtained the same results as Gros and Sollmann.

More recently the subject has been studied at exact pH's by Regnier (1924–1925), Fosdick, Hansen and Dragstedt (1923), and Gerlough (1931). Gerlough tested the duration of anesthesia of the rabbit's cornea when exposed to solutions of butyn in buffered solutions at varying pH. He found that at pH 5.5 to 6.0 very little anesthesia is produced. This furnishes an explanation of the frequently noticed fact that attempts to produce local anesthesia in areas of acutely infected tissue, around acute abscesses, etc., are frequently unsuccessful. Schade (1921–1923), Gaza and Brandi (1926), Habler (1928), and in our laboratory F. P. Light and E. N. Cook (1928) have found that the pus of acute abscesses has a pH of 5 to 6; so that the failure of attempts to induce local anesthesia in such areas could be explained on the basis of pH alone.

Gerlough found that the duration of anesthesia varied directly with the pH (i.e., with the concentration of OH ions).

With procaine he obtained results similar to butyn, except that he found as might be expected that buffered solutions had a slightly more lasting effect that those which had been merely alkalinized to the same degree.

Further confirmation of this was given recently by Gerlough who found that at pH 8.32 one gram of procaine hydrochloride (which has been transformed into free base by the pH) is equivalent to an equimolecular amount of butyn (1.7 gram).

From these studies it would appear that the effect of a molecule of the free base of a local anesthetic is more or less independent of its chemical nature. If this is true it follows that the potency of a local anesthetic salt must be a function of the degree to which it is hydrolyzed, provided that the base is sufficiently soluble to remain in solution or at least in finely divided colloidal suspension. The effectiveness of the anesthesia must also be a linear function of the pH of the tissue.

With other local anesthetics, which are not simple salts, however, alteration of the reaction seems to play little rôle. Sollmann (1918) found that the potency of quinine and urea hydrochloride was unaffected by change of reaction, even in surface anesthesia.

Conversely, of course, the action of acidic local anesthetics like saligenin would be greater in acid media and less on more alkaline media; but all of the substances used so far are too weak acids to manifest this in any marked degree.

Neutral anesthetics like benzyl alcohol and benzoyl carbinol should be unaffected by change in pH of the medium. Trevan and Boock (1927) have shown that the anesthetic effect of benzyl alcohol on the

rabbit's cornea is unaltered by changing the pH of the solution through a range from pH 5 to pH 8.

For clinical use Laewen (1913) recommended a mixture of procaine 0.6 gram, sodium chloride 0.1, sodium bicarbonate 0.15, per 30 cc. H_2O; but Toelkens (1914) found no increased effect, in sacral anesthesia, when $NaHCO_3$ was added to the procaine solutions.

Farr (1923) also found that in practical surgery, chiefly infiltration and nerve block, alkalinizing the solutions had little effect on the results obtained wth procaine.

2. *Adsorption of alkaloid ions.* A part of the action of the anesthetic salt, however, is due not to the splitting off of the free base but to an entirely different mechanism.

Just as its prototype NH_4Cl is ionized into the ions NH_4^+ and Cl^-, the anesthetic salt $R{\equiv}N, HCl$ is ionized to form the ions $R{\equiv}NH^+$ and Cl^-, e.g.,

$$\frac{(\text{Cocaine, } H^+)\ (Cl^-)}{\text{Cocaine, HCl}} = K_{\text{cocaine}}$$

$$\frac{(\text{Procaine, } H^+)\ (Cl^-)}{\text{Procaine, HCl}} K_{\text{procaine}}$$

In the case of weak bases like procaine, cocaine, etc. the degree of ionization is relatively small.

It is easy to demonstrate experimentally, however, that these positively changed ions are selectively adsorbed by negatively charged surfaces such as charcoal, nerve cells, nerve fibers or nerve endings. If one takes a solution of strychnine sulphate which contains a just convulsive dose for a frog, boils it with a well adsorbing charcoal, and filters off the supernatant fluid, this will no longer produce a convulsion when the same amount of fluid is injected into a frog. The positively charged strychnine has been adsorbed on the charcoal.

However, it must be admitted that the amount of ionization of these weak bases is probably much less than the amount which is split off by hydrolysis; and the latter is probably the more important process in local anesthesia.

3. *Effect of variations in the strength of the anesthetic base and of combination with strong and weak acids.* If the free base is the most active constituent two principles should apply which are of importance in the synthesis of new anesthetics:

1. In an homologous series the weaker the base, the greater should

be the amount of free base split off by hydrolysis and the greater should be the anesthetic potency.

2. If the soluble anesthetic salt is made up by combining the base with a weak acid instead of with HCl or H_2SO_4 the degree of hydrolysis will be greater and the anesthetic action should be increased.

Both these principles are in general borne out by the experimental facts, though certain factors tend to modify them:

1. In the deep tissues or the presence of urine, there is a reaction between the anesthetic salt and NaCl which tends to decrease the hydrollysis and to return much of the anesthetic to the form of the hydrochloride regardless of the acid used to form the salt.

2. The anesthetic activity follows the oil/H_2O distribution coefficient, the effect on surface tension of water (Vliet and Adams, 1926), and most especially the effect on the oil/H_2O interfacial tension (Evans and Benedict, 1930–1931) more closely than it does the dissociation coefficient of the base.

The amount of hydrolysis (γ) in aqueous solutions can be calculated from the formula

$$\gamma = \sqrt{\frac{K_w}{K_b C}}$$

where γ is the percentage of hydrolysis, K_w the dissociation constant of water and K_b the dissociation constant of the local anesthetic base. (c.f. H. Taylor, *Treatise of Physical Chemistry*, N. Y., Vol. ii, 825, and W. M. Clark, *Determination of Hydrogen Ions*, Baltimore, p. 337.)

The following basicity constants have been calculated:

Cocaine	2.6×10^{-6}	(Kolthoff 1925)
	4.0×10^{-7}	(Veley 1909)
Procaine	7.1×10^{-6}	(Kolthoff 1925)
Stovaine	1×10^{-7}	(Veley 1909)
Ethylaminobenzoate	6×10^{-12}	(Kolthoff 1925)

If the anesthetic base is combined with a weak acid (acetic, phosphoric, boric acid, etc.) instead of the more usual hydrochloric or sulphuric acid, the degree of hydrolysis is increased according to the formula

$$\gamma = \sqrt{\frac{K_w}{K_a K_b}} = \frac{10^{-7}}{\sqrt{K_a K_b}}$$

where K_a is the dissociation constant of the acid; so that the weaker the acid forming the salt of the weak base the greater the per cent which

is hydrolyzed and, therefore, the greater the concentration of free base present in the solution.

These principles have been given practical application by Copeland and Notton (1925) who found that the boric acid salt of procaine (borocaine) produced more intense and more prolonged anesthesia of the cornea than did the corresponding hydrochloride. Sollmann confirmed these findings for the conjunctiva and on intradermal injection was unable to find any difference in infiltration anesthesia. This has been our experience also. We have also prepared the corresponding boric acid salt of tutocaine, but Wethall was unable to find that this produced any more intense or more lasting anesthesia in the urethra than did tutocaine itself. It is probable that when the anesthetic borate comes in contact with the NaCl of the tissues or the urine, an interchange of ions takes place which by mass action causes much of it to revert to the form of hydrochloride, and that this explains the lack of alteration of effect in infiltration or in the urethra.

4. *Lowering of surface tension and adsorption at interfaces.* As might be expected from the conditions of anesthesia, adsorption of the drug by the nerve fiber or nerve ending is a determining factor in its action. Regnier and Valette (1930) demonstrated that there is a complete parallelism between the degree of adsorbability of cocaine by charcoal under varying conditions and its effects on nerves (see section on basicity, etc.).

Gros (1910) investigated a series of salts of local anesthetics, cocaine, procaine, alypine, stovaine and B-eucaine, from the standpoint of Overton and Meyer's oil/H_2O distribution coefficient, and found that the order of their pharmacological activity runs quite parallel to the oil/H_2O solubility of their bases.

Adams and his collaborators Rideal, Burnett, Jenkins and Dreger (1926) made similar studies on a series of basic alkyl paraminobenzoates similar to procaine from the standpoint of effects on surface tension, oil/H_2O distribution coefficient, basicity and anesthetic action on goldfish. They found that all these substances lower the surface tension of water; and that in general the greater this lowering of surface tension the greater the anesthetic action. But the best parallelism was that between the oil/H_2O distribution and the anesthetic action. In nine out of ten of their substances there was exact parallelism between the order of these two phenomena. These results correspond well with those of King and his collaborators.

King, Hall, Andrews and Cole (1930) have reviewed the Overton

and Meyer theory in a carefully conducted quantitative investigation, determining solubility in water, solubility in paraffin oil and the distribution coefficient. They also determined the effect of the drug on the interfacial tension at the oil/water interface. From these they calculate by Willard Gibbs' formula

$$M = \frac{1}{RT} \cdot \frac{dy}{d\ln c}$$

the actual number of molecules adsorbed at the interface and also the area occupied by the molecules per square centimeter of interface. They find in a homologous series of chemical compounds that

> the calculated adsorption values for the interfaces paralled nearly perfectly the narcotic power of these drugs as measured by their liminal values. . . . . This . . . . seems to indicate that the stronger a drug is adsorbed at the body interfaces between tissues, the stronger will be its narcotic action.
>
> A study of the areas occupied by each molecule in the surface shows that the areas increase fairly regularly as the distribution coefficient decreases and the liminal value increases. Evidently the more powerful a compound the more it will adsorb at the body interfaces and the closer will the molecules be packed in the surface.

They also found that the addition of lecithin to the paraffin oil thus more closely paralleling the composition of a cell surface, greatly increases the oil/H_2O distribution coefficient, as had been shown by Loewe (1913). They find, however, as might be expected, that the adsorption curves for lecithin in oil solutions are sometimes quite irregular, due probably to the fact that lecithin is not a simple solvent but is itself an amphoteric compound, readily hydrolyzed into choline, phosphoric acid and glycerine each of which would act as an additional factor in modifying the results. However, these would only tend to increase the adsorption at the interface disproportionately, and both lecithin and kephalin are, therefore, most important components of the cell for increasing the action of narcotic drugs.

5. *Diffusion and penetration.* Along somewhat similar lines, Jumikura (1925) showed for local anesthetics that procaine diffuses rapidly into a gelatin gel into which lecithin has been incorporated; and that the amount of substance absorbed by the gel is fairly parallel to the local anesthetic potency. Cocaine, totocaine, alypine and eucaine are taken up by the gel more strongly than is novocaine.

Wertheimer and Paffrath (1925) have found similar relations between the rate of action and diffusibility in other substances. For example,

the pharmacological action in a series of choline derivatives, histamine and pilocarpine varies inversely as the rate of diffusion through frog's skin or through the wall of the intestine. It is probable that this factor may explain some of the synergistic actions discussed below.

6. *Effects of duration of application and of concentration.* As might be expected the anesthetic effect varies with both the time that the drug remains in contact with the nerve, and with the concentration of the solution used.

Gerlough (1931) allowed solutions of butyn buffered to a constant pH 6.97 to remain in contact with the rabbit's cornea for varying lengths of time, after which he washed the conjunctival sac out completely with 0.9 per cent NaCl solution. He found that the duration of the anesthesia, A, varied very exactly with the square root of the time, t, during which the drug came in contact with the cornea. $A = k\sqrt{t}$

Sollmann (1918) has determined the rate of absorption of cocaine hydrochloride from the urethra. When his results are recalculated by the same formula, they accord fairly well with the findings of Gerlough.

TIME IN URETHRA	PER CENT ABSORBED	$A = k\sqrt{t}$ (Recalc.)
minutes		
10	42.6	11.6
20	42.7	9.7
30	59.7	10.8
40	61.0	9.7
Average		10.4

Laubender (1928) found that the time elapsing before the onset of anesthesia varied inversely as the logarithm of the concentration of the anesthetic; and Gerlough (1931) obtained similar results for the duration with procaine, procaine borate, butyn and butyn borate. In other words, the anesthetic effects follow the equation for adsorption phenomena (Freundlich's adsorption isotherm) as might be expected from the nature of the process of anesthesia.

It must be admitted, however, that the results of Sollmann, and those in his tabulation from other authors, upon the duration of anesthesia produced by various local anesthetics injected intracutaneously into the human skin, when recalculated, do not show any accord with this formula. In intracutaneous injections the results are also affected by

the fact that the local anesthetic remains in situ for a considerable time, by the rate of absorption of the drug from the site of injection, and by a number of other additional complicating factors which are absent from Gerlough's experiments. The latter probably more nearly portray the effects of this single variant.

One must also realize that Sollmann's experiments were carried on from a different standpoint, and, therefore, would not furnish data as well suited to this interpretation as Gerlough's.

Gross (1910) found for frog's nerves and Gross and Hartung (1911) for anesthetic toxicity on tadpoles that the threshold molar concentrations of the bases of cocaine, alypine, B-eucaine and procaine are relatively constant throughout the series $\frac{M}{3200}$ to $\frac{M}{1600}$, and that the concentration which produces anesthesia in tadpoles is about half that which produces motor block in the frog's nerve or intradermal anesthesia in man.

C. *Synergistic actions.* 1. *Synergism with epinephrine.* Braun (1903) demonstrated the remarkable fact that epinephrine added to local anesthetics (cocaine, procaine) greatly increased the duration of anesthesia and also lowered the threshold concentration (i.e., the minimal concentration which would produce local anesthesia). This effect can be produced even with 1:100,000 solutions of epinephrine though 1:50,000 is the more usual for dental and surgical anesthesia. This occurs in spite of the fact that the local ischemia produced by epinephrine itself causes a definite local acidosis (Rous and Drury, 1925) which, as stated above, would tend to diminish the anesthesia.

On the other hand, ephedrine has no such marked synergizing action and only slightly prolongs the anesthesia, probably because when mixed with local anesthetics it produces a local hyperemia rather than a local anemia. Chen (1928) and Chubyung, Pak and Read (1928) and Hamet (1931) have shown that cocaine diminishes the blood pressure raising action of ephedrine and this may also apply to other local anesthetics.

2. *Synergism of potassium salts and of local anesthetics.* M. L. Menten (1912) found that when nerves were immersed in dilute solutions of some salts of potassium they lost their powers of conduction. Menten obtained these effects with potassium sulphate but not with potassium acetate, citrate, oxalate or tartrate; and no effect was obtained with magnesium salts until a concentration of 3.1 per cent (about $\frac{m}{8}$) was

reached. Sodium and lithium salts did not produce in ordinary concentrations blocking of the nerves.

Kochmann, Zorn and Hoffmann (1912–14) proceeded to put this property to use in increasing the potency of solutions of local anesthetics to be used in practical surgery. On intracutaneous injection in man they found that the addition of K_2SO_4 definitely increases and prolongs the local anesthetic action of cocaine and of novocaine (to somewhat less than double the usual effect), tropacocaine is slightly increased, acoine about unchanged, and on the other hand the action of alypine and stovaine is definitely diminished. For optimal results they dissolved the salt of the anesthetic in 0.4 per cent K_2SO_4. They believe the K_2SO_4 retards the absorption of the local anesthetic in the surface of the nerve, and in support of this, state that they failed to get potentiation when they used KCl. However, they also mention the occurrence of edema about the site of the injection, which probably indicates some injury of the neighboring blood vessels and tissues. This probably accounts for the fact that the addition of K salts to local anesthetics has not come into general use in practical surgery. H. Braun (1913) obtained optimal effects with a solution of procaine 4 per cent in 0.4 per cent K_2SO_4 to which enough epinephrine is added to make $\frac{1}{50{,}000}$ concentration.

For nerve block anesthesia in dental practice Adloff has used procaine in 1 per cent K_2SO_4 as he found that 0.4 to 0.5 per cent did not potentiate when used in this way. Hoffmann and Kochmann (1912–14) regard this strength of K_2SO_4 as too great and believe that potentiation should be obtained by the addition of epinephrine to the solution.

In partial accord with these observations on man, Sollmann (1918) found that potassium chloride did not increase the anesthetic action of cocaine, novocaine, tropacocaine or quinine and urea on the sensory fibers of the frog's sciatic plexus, the frog's skin or the rabbit's cornea, though he did find that this salt increased to about eight-fold their blocking effect upon the motor fibers. He did not use potassium sulphate, with which Kochmann and his collaborators obtained their best effects.

Hoffmann and Kochmann (1914) also found that the mixtures of potassium sulphate and local anesthetics were less toxic to guinea pigs on intravenous injection than was the solution of the local anesthetic which showed the same degree of anesthetic action upon intradermal injection in man.

However, Tar (1916) states that 10 to 15 per cent magnesium sul-

phate injected into the vicinity of nerves lessens pain without impairing motor functions.

Schonfeld (1920), Berger (1924) and Hlarowitz and Bialinski (1931) claim that $MgSO_4$ is still more potent than K_2SO_4, though this is not in accord with the experience of the earlier workers.

The synergizing effect of the K salts is somewhat difficult to explain theoretically. It is possible that the potassium first increases the permeability of the surface layer of the nerve sheath or nerve ending, and thus facilitates the entrance of the anesthetic.

3. *Synergism with methylene blue and purines.* Lipschitz, Laubender and Weingarten (1928) have shown another type of synergism which might be explained upon the same basis, and which may be capable of clinical application, especially in spinal anesthesia. Basing their experiments upon Ehrlich's (1886–90) demonstration that methylene blue stains sensory nerve endings selectively and also exerts an analgesic effect, they found that preliminary immersion of the frog's skin in methylene blue causes a synergistic increase in the effect of local anesthetics. They found further that salts of caffeine and theobromine had the same effect, hastening the onset of anesthesia from (in the order of potentiation) alypine, cocaine, procaine. They found that these drugs form loose combinations with caffeine with an affinity which is exactly the reverse order. Theobromine compounds had the same effects as those of caffeine. Since caffeine and theobromine increase the penetrability of the meninges and of most membranes in the body it would seem probable that their effect was exerted in the same general way as that of the potassium salts.

4. *Synergism with narcotics and antipyretics.* On the other hand, addition of narcotic and sedative drugs to the local anesthetic solutions can also potentiate their effect (Esch, 1910) though this may be masked by the irritating effect of the former. v. Issekutz (1912) found that addition of antipyrine increased the anesthetic power of cocaine, B eucaine and procaine on the frog's skin. Stender and Amsler (1929) found that antipyrine and lactophenine synergize cocaine in the rabbit's cornea, but failed to produce any increase in the action of procaine. Kochmann and Hurtz (1923) demonstrated that morphine synergized the motor blocking effect of cocaine. We have also found that a solution of procaine hydrochloride made up in a saturated solution of acetyl salicylic acid (0.3 per cent) produces anesthesia of much longer duration than the procaine alone, though the onset of this is preceded by intense pain which also lasts for many hours after the subsidence of the anes-

thesia. In this regard the effect of acetyl salicylic acid resembles that of other phenolic esters as found by Jensen (1925), and, therefore, cannot be used in practice.

5. *Synergism with proteins.* Shillito (1929) has made the remarkable observation that the addition of egg albumin and gliadin to local anesthetics increases their action markedly. This same observation was made independently on the rabbit's cornea by Stender and Amsler (1929) for fresh egg white but they found no potentiation by the addition of guinea pig serum, rabbit's serum, gelatin or tragacanth, nor by ordinary crystalline (?) egg albumin. Schmidt (1930) has confirmed Shillito's findings both on the cornea and on intraspinal injection. It seems probable that this potentiation may explain some of the good results obtained by Pitkin and others with spinal anesthesia produced by "spinocaine" in which Pitkin added an altered gliadin to the procaine in order to regulate the specific gravity of the anesthetic solution.

Sollmann (1919) has shown that procaine and the other soluble local anesthetics can precipitate or at least cause an opalescence in serum, and this factor may play a rôle in bringing about the synergizing action either by an adsorption phenomenon or by a true chemical combination.

METHODS OF TESTING EFFICACY OF LOCAL ANESTHETICS. The potency of local anesthetics may be determined experimentally in several different ways, none of which furnishes all the information required for clinical use. Rider (1930) very correctly criticizes the lack of uniformity in the procedure of different investigators, since this prevents the quantitative comparison of the results recorded in the literature or any reduction of the general findings to mathematical formulas. There is certainly a crying need for the development of standardized methods which will be adhered to by all investigators.

The local anesthetics are usually tested for their effects upon:

1. The mucous membrane of the human tongue
2. The frog's sciatic nerve, motor and sensory
3. The frog's skin
4. The frog's spinal cord
5. The rabbit's cornea
6. The intradermal wheal in the guinea pig's skin
7. The intradermal wheal in the human skin
8. The production of anesthesia, as shown by paralysis, in goldfish, tadpoles, etc.

The best comparisons of various methods by individual authors are given by Sollmann (1918), by Gros (1929) and by Munch (1931).

Mucous membrane of the human tongue. The oldest method of testing local anesthetic action is the deadening of sensation on the mucous membrane of the tongue by direct application upon the tip (Wöhler, 1860), but as very little absorption takes place from this mucous membrane only cocaine and butyn produce anesthesia when applied to it, and most of the other cocaine substitutes are quite ineffectual. Though pain and taste sensations are deadened, sensation to pressure remains.

Squibb (1892) attempted to make this method quantitative by allowing a series of solutions of cocaine hydrochloride of known concentrations to dry upon strips of filter paper of definite size. He cut these papers into small bits of definite area, so that each bit contained a known amount of cocaine, and when placed upon the human tongue would cover a definite area. He obtained anesthesia in one person with $\frac{1}{4000}$ grain cocaine hydrochloride, another required $\frac{1}{2000}$.

Sollmann (1919) developed a method for testing insoluble local anesthetics more or less quantitatively on the gums. He diluted them by mixing them with varying proportions of talcum powder and applied the mixture to the gums. This is a rapid semiquantitative method for comparison of relative efficiencies. The sensitivity of the gums was tested by touching them with the tip of a lead pencil.

Mammalian sciatic nerve. Witzel (1886), Kochs (1886) and Feinberg (1886) exposed the dog's sciatic nerve, applied cocaine solutions to it and demonstrated the development of the sensory block.

Mosso (1890) produced motor paralysis on dog's phrenic nerve after application of 10 per cent cocaine.

Van Leeuwen and Verzar (1923) used the rabbit's sciatic nerve, exposed 2 cm. of the nerve on both sides under deep anesthesia, slipped around it a short bit of slit rubber tubing, plugged the ends carefully and tied it in place. The animals were then decerebrated, artificial respiration started and the body temperature maintained. An hour after the cessation of the ether anesthesia the solution of the drug to be tested was poured into the chamber around the nerve, the standard local anesthetic similarly applied to the nerve on the opposite side. The nerve was then stimulated with electrodes below the block, and the stimulus which just caused a stretching reflex in the peroneal muscles determined. As soon as stimuli fail to cause reflex responses the solution is washed out with physiological salt solution. Five-tenths per cent procaine solution gave complete anesthesia in 36 minutes.

Schmitz and Loevenhart (1924) used the sciatic nerve of the rabbit, exposing it under ether anesthesia throughout its entire length, sec-

tioning it as far distally as possible and then stimulating the central end. The anesthesia was kept uniform and as light as possible. The respiration was recorded and the minimal stimulus from an induction coil which would produce a change in respiration was determined. They then introduced ½ to 1 inch of the severed nerve into the anesthetic solution, left it there for exactly one minute, and upon removal covered the end with gauze saturated with warm 0.9 per cent NaCl. (Obviously, it would have been better to have washed off the nerve by several immersions in warm salt or Ringer-Locke's solution.) The response of the anesthetized end was then compared with that of the unanesthetized portion above.

Frog's skin (Turck's method). Since the frog's skin is an absorbing surface, somewhat similar to the conjunctiva, anesthesia can be determined by first immersing the frog's foot in the solution of the anesthetic, washing this off, and then immersing the foot in dilute acid or stimulating the skin with electrodes. It is essential to determine the interval elapsing before the onset of nerve block, the time during which the drug is applied, the duration of the block after anesthesia, and the intensity of the stimulus which elicits the response. Most investigators have used 1 per cent HCl or 1 per cent H_2SO_4 to furnish the irritating stimulus to the skin, but Lipschitz and Weingarten's method (1928) of testing the threshold with graded concentrations of acid may be used.

Frog's sciatic. Alms (1886) was perhaps the first to expose the frog's sciatic nerve and to demonstrate both motor and sensory block upon the application of cocaine solutions. The cerebrum, midbrain and medulla should be either cut off or completely destroyed by pithing. About 2 cm. of the nerve should be exposed and the drug applied to it, either by simply dropping the solution into the gutter formed between the muscles or by applying to the nerve a wisp of cotton soaked with the drug. If the duration of the action is to be observed, as it should be, the drug, nerve and intermuscular fossa are thoroughly washed out with salt solution. The time during which the drug has been in contact with the nerve should be carefully noted since Gerlough (1931) has shown that duration of anesthesia varies with the square root of the time of the contact of the drug with the nerve.

Kochmann and Boehminghaus (1928) have perfected this method by placing under the nerve a small oblong piece of rubber dam with nicks cut at opposite sides so that the nerve fits into the nicks. Over this and under the nerve they place a second small bit of rubber dam. By so doing they have the nerve lying in a little rubber trough in which they

can place the anesthetic solution, and wash it out without getting any into contact with the muscles, skin or other tissues.

Frog's sciatic plexus. Sollmann (1918) obviated the danger of injuring the nerve by handling, by applying the drug directly to the sciatic plexus on one side within the abdomen, after excising the abdominal viscera. Rider (1930) propped up the opposite side of the abdomen a little so that the anesthetic solution gravitated to the side to be tested, and the opposite side remained as a control. This is in many respects a more satisfactory method than that of exposing the nerve. It eliminates the factor of trauma.

The onset of anesthesia is obtained by determining the time at which the frog no longer responds by reflex contraction of the limb or the opposite leg upon the application of a definite stimulus below the point at which the drug is applied. For most investigators this stimulus is the irritation of the cutaneous nerves, produced by dipping the frog's foot into 1 per cent HCl or H_2SO_4 which is then promptly washed off with dilute $NaHCO_3$. Lipschitz and Weingarten (1928) used immersion into a series of dilutions of HCl 0.0100, 0.0125, 0.0150, 0.0175, 0.0200, 0.0300, 0.0400 M in order to establish quantitatively the threshold of stimulus and degree of anesthesia, as well as the complete disappearance of all reflex activity.

Some investigators use direct stimulation of the skin of the foot, others direct stimulation of the exposed sciatic nerve below the blocked area.

It is most important to determine both the interval required for the onset of anesthesia, the time during which the drug is applied to the nerve and the period during which anesthesia continues after the drug has been removed, and the nerve has been washed off with 0.6 per cent NaCl.

Effects on motor conduction are determined in the usual way by applying electrodes carrying a faradic current from an induction coil directly to the exposed sciatic nerve above the area to which the drug is applied. If possible the strength of stimulus in electrical units should be given.

Spinal anesthesia in the frog. The skull and brain of the frog are cut away exposing the spinal canal, the animal is suspended by the lower jaw, and the normal reflex time tested. One drop of the local anesthetic solution is introduced on one side of the spinal cord and the reflex is determined from time to time (Braun, Sollmann and Hanzlik, 1928).

Essex and Lundy (1931) injected 0.05 to 0.1 cc. of the drug by cister-

nal puncture into the frog's brain case, taking care not to insert the needle into the brain or spinal cord. The rapidity with which the drug becomes immobilized and the duration of immobility are taken as the indices of anesthesia.

Bieter, Harvey and Burgess (1931) have improved this method by injecting the solution directly into the spinal canal of the frog. The spinal column is bent ventrally at a 90° angle at the level of the iliac crest. The needle is then inserted, pointing caudad, just proximal to the upper edge of the urostyle which can be palpated through the skin, and it then comes to rest just inside the urostyle. The anesthetic injected thus bathes the cauda equina and by means of varying the volumes of fluid injected, the solution can be made to rise in the spinal canal. (See p. 260, *Spinal Anesthesia.*)

Rabbit's cornea. One of the oldest and most satisfactory media for testing local anesthetics is by the effect upon the rabbit's cornea when the solution is instilled into the conjunctival sac. This was used by Koller (1884) in the classical experiments which led to the introduction of cocaine into ophthalmological practice. In order to obtain accurate data for most purposes the solution should be promptly and completely washed out of the conjunctival sac with 0.9 per cent NaCl solution after a definite length of time, and the time of both onset and duration of anesthesia should then be recorded.

The anesthesia is usually observed by touching the cornea with the dull point of a pencil or the rounded end of a thin glass rod. Absence of the winking reflex is taken as the evidence of anesthesia. It is most important, of course, that the point used to touch the cornea should be brought forward along the side of the animal's head, so that it does not see it approaching before the cornea is touched. Sollmann (1918) found the rounded point of a pencil excellent for carrying out this test. Hirschfelder and Abraham have made a very simple modification of this apparatus, which is easily constructed at no cost whatever and is quite accurate. Their esthesiometer consists of a strip of ordinary photographic film about 10 cm. long and 1 cm. wide, the free end of which is tapered to a sharp point upon which a minute ball of sealing wax or de Khotinsky cement is melted. The broad end of the strip of film is fixed at right angles to a piece of card; and a line is drawn upon the card to show the position taken by the film when at rest. By pressing the free end against the balance pan of a chemical balance the angular bending of the film produced by weights of 10, 20, 30, 40, 50, 100, etc. milligrams placed on the weight pan of the balance can be measured

and marked off on the card. The point can then be pressed against the rabbit's cornea and the amount of pressure necessary to induce a wink reflex can be determined. By this means the stages of gradual onset and gradual subsidence of anesthesia can be studied. Further use of apparatus of this type would probably add considerably to the available quantitative data upon the rate of onset and subsidence of local anesthesia.

Coles and Rose (1929) call attention to the fact that on the cornea irritative effect is as important as anesthesia. They, therefore, note and record the degree of capillary engorgement, of the palpebral conjunctiva, reddening of the sclera, corrosion of the superficial layers over the cornea, pitting and crack production in the cornea, and the production of shreds of mucus. Absence of all these are important for obtaining a non-irritant local anesthetic. They find that tutocaine produces slight engorgement and slight pitting; cocaine, slight engorgement and severe pitting; and butyn severe engorgement and severe pitting, while two piperidine derivatives of cinnamic acid (see p. 219) do not produce any of these effects.

Tatum (1931) has also used anesthesia of the rabbit's cornea as a medium for testing nerve blocking effects. For this purpose he injects the anesthetic solution into the subcutaneous tissue about the external canthus of the rabbit's eye by inserting the needle through the skin one or two centimeters back from and lateral to the eye. One half to 1 cc. is injected causing the tissue to bulge slightly.

Intracutaneous injections in mammals. Pittenger (1921) tested anesthesia on wheals made by subcutaneous injections on the backs of dogs. Meeker (1925) showed that the subcutaneous tissues are not sufficiently sensitive and substituted intracutaneous infiltration. Rose (1929) calls attention to the variation in sensitivity of the skin on the backs of dogs, and substitutes intracutaneous injection of 0.1 cc. of the solution into the backs of guinea pigs after clipping the hair. The area is marked off with ink and the skin stimulated with electrodes attached to a Harvard inductorium connected with two dry cell batteries in parallel. The stimulus which is just perceptible when applied to the flexor surface of the human forearm is usually just sufficient to cause the guinea pig to move.

Human intracutaneous anesthesia. Anesthesia of the skin of the human forearm after intracutaneous injection of a small quantity (0.3 to 0.5 cc., usually 0.4 cc.) of the solution of the drug was introduced by Braun (1905) and since then has come into general use, especially in

the hands of Hoffmann and Kochmann (1914) and Sollmann (1918). The skin on the flexor surface of the forearm is shaved, washed with alcohol or ether, and the drug introduced into the skin, not subcutaneously, through a fine hypodermic needle. If dilute solutions are used they should be made in 0.9 per cent NaCl. Sensation to light pressure is best tested by means of a wisp of cotton drawn out to a fine point. The best method is to determine the minimum concentration which produces anesthesia; though the duration of anesthesia should be noted also. In the latter case, the absorbability of the drug as well as its anesthetic power comes into play, but this in itself may be of practical importance.

Anesthesia of goldfish. Adams, Rideal, Burnett, Jenkins and Dreger (1926) determined the time necessary to anesthetize goldfish when placed in solutions of a number of local anesthetics, and found this a satisfactory method for obtaining comparative and even quantitative results. Their method has been slightly modified and improved by Dailey and Benedict (1929) who used 2 to 4 gram goldfish weighed in a wire strainer, and carefully kept in water at 20°C. ±1° for 48 hours. These are then placed in a 600 cc. beaker containing 250 cc. of the local anesthetic solution made in plain distilled (not doubly distilled) water. The temperature should not vary more than 0.1° from 20° during the experiment. The anesthetic produces at first a stage of excitement, during which the fish moves violently; it then becomes relaxed but may still react when the tail or fins are pressed with a glass rod. Disappearance of this reaction is taken as the point of anesthesia. They used forty goldfish for each determination, and found the average anesthesia time 12.4 minutes for 1:5000 cocaine hydrochloride, mean deviation 1.4 minutes, maximum deviation 3.6; for 1:5000 procaine hydrochloride they found average anesthesia time 14.7 minutes, mean deviation 1.6 minutes, maximum deviation 4.7 minutes. For the goldfish cocaine hydrochloride is 8.4 times as anesthetic as procaine but somewhat more toxic (0.2 per cent vs. 1.5 per cent respectively). For mammals cocaine is from six to ten times more efficient than procaine.

General conclusions regarding methods of testing. McGuigan (1924) has called attention to the fact that the results obtained with the same anesthetics tested by different methods vary very widely. For example, he obtained for $\frac{\text{procaine}}{\text{cocaine}}$ ratio the following: Frog's skin, 0; frog's sciatic, 1/16; rabbit's cornea, 3/16; dog intracutaneous, 1/11.

It is most important, therefore, when giving data on local anesthetics to state the method used and the results with each.

The clinical effectiveness of a drug will also be found to vary considerably in different methods of surgical procedure. For example, phenacaine (holocaine) is quite useful when applied to the human conjunctiva but of little use elsewhere. Saligenin is quite satisfactory for infiltration anesthesia of not too long duration but is useless for nerve block and for spinal anesthesia. Similar variations will be met with in other drugs; and it is important, therefore, to learn the field of clinical practice for which each is best adapted.

THERAPEUTIC COEFFICIENT. In order to determine the value of an anesthetic in practice both the anesthetic power and the toxicity, and the ratio between them must be taken into account. Ehrlich termed this the Therapeutic Coefficient $= \dfrac{\text{Minimum Lethal Dose}}{\text{Minimum Therapeutic Dose.}}$

Hoffmann and Kochmann (1914) calculated the Anesthetic Value (W) as follows:

k = Minimum anesthetic concentration (in percentage) for human intracutaneous injection

L = Minimal subcutaneous lethal dose (gm. per kilo) for guinea pigs

$$W = \frac{1}{K} \times L$$

and also

$$W = \text{active value} = \frac{1}{K} L Z$$

where Z is the duration of anesthesia.

Schmitz and Loevenhart (1924) made a similar calculation from the product

Therapeutic Coefficient = Duration of anesthesia (in minutes) × Minimum lethal dose for white mice in milligrams per kilo

They then took the figures obtained for cocaine (4200) as the unit, and determined the cocaine ratio,

$$\text{Ratio to cocaine} = \frac{\text{Therapeutic coefficient for drug}}{\text{Therapeutic coefficient for cocaine}}$$

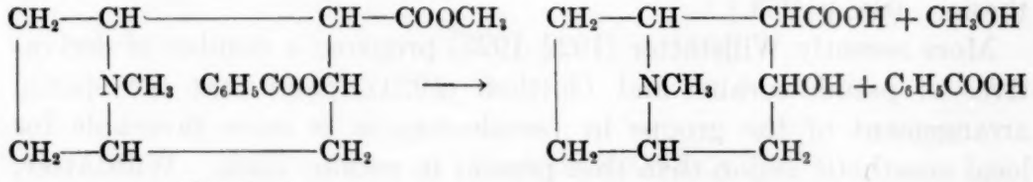
Since many of the articles in the literature give ratios compared with cocaine one can conveniently use the following ratio:

$$\text{Therapeutic value of drug} = \frac{\text{Ratio to cocaine (for anesthesia)}}{\text{Ratio to cocaine (for toxicity)}}$$

For any individual series of observation this new ratio gives a therapeutic coefficient somewhat similar to those of Hoffman and Kochmann and Schmitz and Loevenhart, but the data so obtained cannot be compared with other numerical data in the literature because hardly any two observers have based their calculations on the same method of recording anesthetic power and of determining toxicity. As McGuigan (p. 215) has shown, such data even when obtained with the same drugs vary greatly for different methods of testing.

Vliet and Adams (1926) and Evans and Benedict (1930–31) have compared the potency of several local anesthetics with their physical constants, i.e., dissociation coefficient of the anesthetic base and effect of the anesthetic salt on lowering of surface tension of water. They find that the order of anesthesia parallels that of lowering the surface tension more than it does the basicity. Evans and Benedict have found still further that this parallels the diminution of surface tension at the interface between oil and water more closely than any other single factor; so that the latter might often be taken as a rough measure of anesthetic activity in prophesying the action of new substances.

CHEMISTRY OF LOCAL ANESTHETICS. The early studies of the structure of cocaine led to the development of substitutes for the drug. Wöhler (1862) and his pupils Niemann and Lossen found that on heating cocaine with HCl it was split to yield benzoic acid and a new organic base which he termed "ecgonine." Lossen (1865) found that methyl alcohol also is split off, and concluded that cocaine is methyl benzoyl ecgonine. Later studies showed that ecgonine itself is a piperidin derivative, a fact which has played an important rôle in the synthesis of substitutes for cocaine.



Cocaine[4]

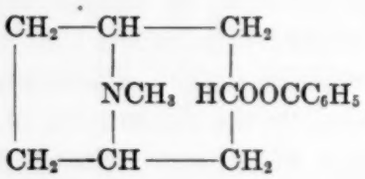
1—normal methyl benzoyl—ecgonine.

[4] Unless otherwise specified the local anesthetics are used in the form of the hydrochlorides, and the formulas given are those of the anesthetic bases.

Filehne (1887) found that if the methyl alcohol group is split off from the cocaine molecule the substance loses most of its local anesthetic action; and Ehrlich (1890) also established the "anesthesiophoric" action of the benzoyl group, as well as the toxic action of the benzoyl ecgonine and the ecgonine residue. The absence of anesthetic power in ecgonine and benzoyl ecgonine is probably due to the fact that these acid substances readily form water soluble sodium salts which are but slightly soluble in lipoids.

More extended studies upon the chemistry of cocaine and cocaine-like compounds will be found in the papers of Einhorn (1888–1889) and the pharmacological studies of Poulssen (1890).

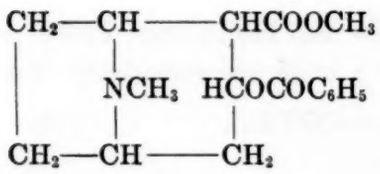
On the other hand tropacocaine isolated by Giesel (1891) the alkaloid which is found in Javanese coca,



Tropacocaine
(Benzoyl—ψ—tropeine)

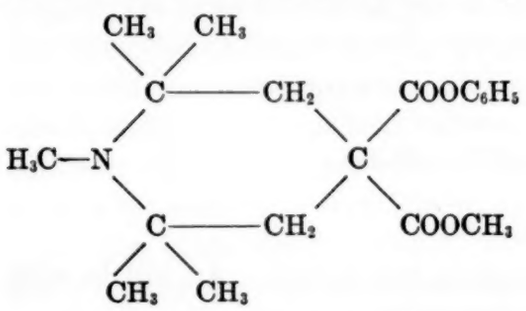
was synthesized by Liebermann (1892) and found to be benzoyl pseudo tropeine, with a base resembling that present in atropine. It differs from methyl ecgonine only in having a CH_2 group in place of the CH COOH group on the ring. But this tropeine derivative is much more soluble in lipoids than the sodium salt of benzoyl ecgonine and tropacocaine is, therefore, an excellent local anesthetic, and is somewhat less toxic than cocaine. So that before the introduction of procaine tropacocaine was the most valuable local anesthetic especially in spinal anesthesia. (See p. 253.)

More recently Willstätter (1922–1923) prepared a number of derivatives of pseudo-cocaine and Gottlieb (1923) found that the spatial arrangement of the groups in pseudo-cocaine is more favorable for local anesthetic action than that present in cocaine itself. Willstätter, Wolfes and Maeder (1923) accordingly prepared "psicaine" (d-pseudococaine acid tartrate) an isomer of cocaine characterized by cis-trans isomerism whose anesthetic power is greater than that of cocaine. (See p. 262.)

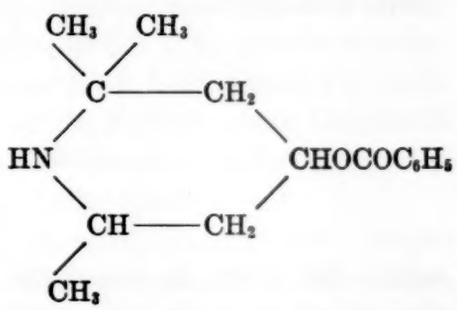


Psicaine
(d—ψ—cocaine)

The demonstration of the piperidine group in cocaine and ecgonine and of Ehrlich's "anesthesiophor" action of the benzoyl group soon led to the introduction of the benzoyl piperidine compounds eucaine A (Vinci 1896) and B (Vinci 1897).

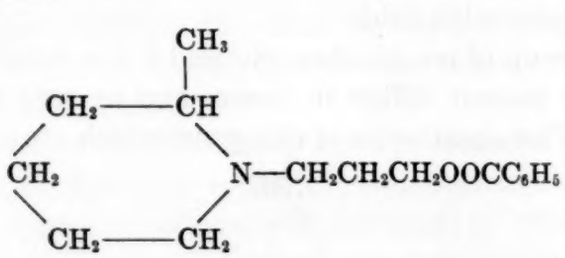


Eucaine A



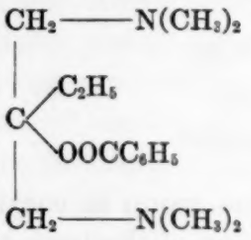
Eucaine B

The first of the important cocaine substitutes to have widespread use in practice, they were good local anesthetics, even for mucous membranes and much less toxic than cocaine but had a tendency to cause hyperemia and local edema in the tissues. These substances have been supplanted by the anesthetics of the procaine series, but interest in this type of compound has been revived by the work of McElwain (1926–1929), Marvel and Shelton (1929) and Blicke and Blake (1931) upon piperidine and pyridine derivatives, the most interesting of which is McElwain's "neothesine."

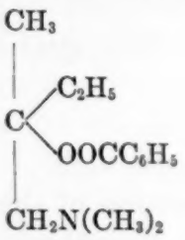


Benzoylation of other basic substances was soon tried and these investigations resulted in the development of the practical local anes-

thetics alypine (Impens and Hoffmann, 1905), and stovaine (Fourneau, 1904) which still enjoy a wide use especially in urethral anesthesia.

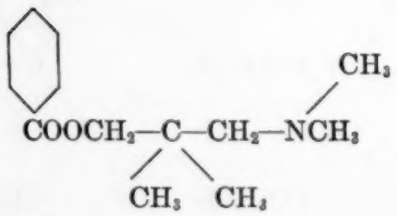


Alypine



Stovaine

Fourneau and Samdahl (1930) have prepared another substance isomeric with stovaine,



which has twice its anesthetic power on the cornea. As will be seen, this substance is also very similar to larocaine.

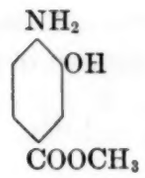
The greatest of all advances in the synthesis of substitutes for cocaine was made when A. Einhorn (1892–1910) synthesized a large series of esters of aminobenzoic acid. He first found that the ordinary esters of aminobenzoic acid (methyl, ethyl, propyl, iso-butyl, etc.) are capable of anesthetizing exposed nerve endings with which they come in contact and are, therefore, useful as dusting powders or in ointments for relieving the pain of ulcers, burns, the inflamed mucous membranes, raw surfaces after hemorrhoid and other operations, etc. However, they are practically insoluble in water, and unlike any of the other basic substances used as local anesthetics, they are too weak bases to form water soluble salts with acids.

The amino group of p-aminobenzoic acid is too weakly basic for this. Therefore, they cannot diffuse in tissues and be used for infiltration or nerve block. The anesthetics of this group which are in practical use are

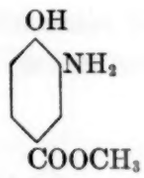
Ethylaminobenzoate (U.S.P.)
(Anesthesine)

Propaesine

N—butyl ester Butesine
(also Scuroform)
iso-butyl ester Cycloform



Orthoform



Orthoform New

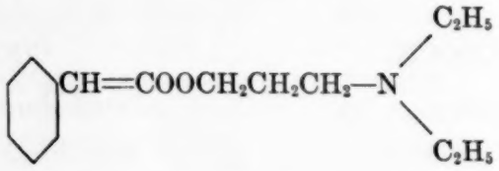
In these insoluble substances lengthening of the side chain produces but little effect on anesthetic power. Sturmes and Luders (1908) found that, the N-propyl (propaesine) is strongly anesthetic when applied to the mucous membrane of the mouth, the iso-propyl and iso-butyl, weaker; the iso-amyl strong but irritating. Sollmann (1919) determined the threshold concentration by diluting the powder with talcum powder. He found the ethyl, n-propyl and iso-butyl ester have about the same effectiveness on the rabbit's cornea and on the human gums (threshold concentration 2.5–5.0 per cent). Brill (1921) found no difference on the tongue between the n-butyl, iso-propyl and allyl esters.

On the other hand, Adams, Rideal, Burnett, Jenkins and Dreger (1926) investigating the effectiveness of these compounds by the much more delicate method of testing anesthesia on the goldfish found very marked differences, which are quite in accord with the general laws of increasing length of side chains in other compounds.

With the normal esters they found the molar threshold concentration to range for ethyl M. 0.00042, ethyl 0.00013, n-propyl 0.00004, n-butyl 0.00002, N-amyl 0.000022. This almost exactly accords with the general law of Richardson (1869), Traube (1913) and Kamm (1921), that the addition of each CH_2 to the molecule trebles its activity. They found iso-butyl less active than n-butyl though reduced by more than the halving of the effect which usually holds for iso in comparison with n-compounds. Adams and his collaborators also found a very definite correspondence between the effect on the lowering of surface tension of aqueous solutions, the oil/H_2O distribution coefficient and in the anesthetic effect on goldfish.

Lowsley, Angle and Hooper (1924) have produced soluble compounds of these otherwise insoluble esters of aminobenzoic acid by heating them in strong syrups of glucose (40 per cent) or other aldose sugars. They found that these solutions which are very low in toxicity as compared with other local anesthetics can be used for infiltration anesthesia and especially for anesthesia in the urethra. Their instability, however, has prevented them from coming into general use.

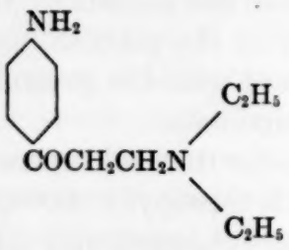
Basic ester of cinnamic acid (procaine series). A basic ester of cinnamic acid "apothesine" has been in use for a number of years.



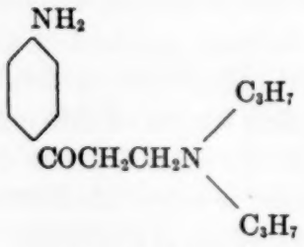
Apothesine

It is slightly less powerful than procaine, somewhat slower in action and about twice as toxic.

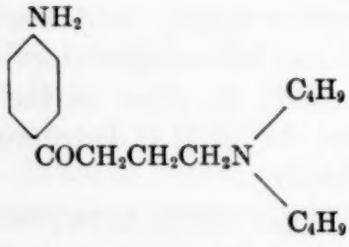
After preparing the simple esters of para aminobenzoic acid Einhorn proceeded to make others which would form water soluble salts, by esterifying with basic alcohols such as di-ethylamino alcohol, etc. In 1905 he introduced procaine (novocaine) the most useful of all the local anesthetics, and by 1910 he had prepared and studied a large series of similar compounds. The substances of this type are the local anesthetics most in use at present.



Procaine (U.S.P.)
(Novocaine)



Isocaine
Di-iso propyl ethanol
aminobenzoic ester



Butyn
(as the sulphate)

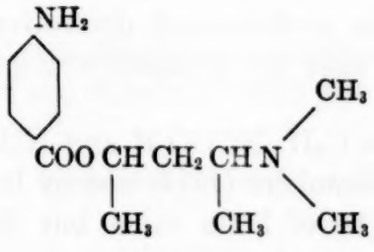
Schmitz and Loevenhart (1924) have shown for this series that both anesthetic action and toxicity increase with lengthing of the side chains and Fenwick, Florence and Gilman (1929) have found that the local

anesthetic effect increases with the strength of the base, though Adams and his collaborators (1926) and Evans and Benedict (1930–31) have found that this is not invariable.

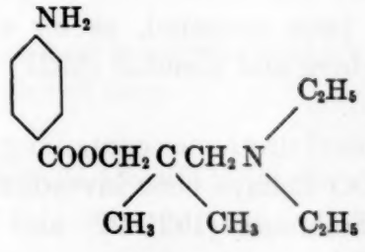
Vliet and Adams (1926) found in this series that when R is lengthened in the $-N\begin{matrix}R\\R\end{matrix}$ complex the basicity (K) decreases progressively. This corresponds to an increased hydrolysis of the anesthetic salt and a corresponding increase in potency (see above p. 202). Most isocompounds are more strongly basic than the N— compounds though the iso-butyl is an exception.

Increasing the length of the chain between the $NH_2C_6H_4CO$ group and the NR_2 group has the exact opposite effect on basicity; though Schmitz and Loevenhart (1924) showed for the n-propyl compounds that increasing this part of the chain increases its toxicity from M.L.D. 550 mgm. per Kilo for white mice for the $-CH_2CH_2N-$ to M.L.D. 200 mgm. for the $-CH_2CH_2CH_2N-$, and for the N-isopropyl compounds from M.L.D. 400 mgm. to M.L.D. 150. These increases in toxicity conform fairly well to the law of Richardson, Traube and Kamm; but according to the findings of Schmitz and Loevenhart there is very little if any increase in the anesthetic power of the drugs upon the rabbits to correspond to the observed increase in toxicity. It would appear, therefore, that from the standpoint of anesthetic potency the ethanol represents the optimal toxicity length of this part of the side chain.

On the other hand, branching of this part of the side chain causes the basicity again to decrease (Vliet and Adams) and this is again accompanied by increase in both anesthetic potency and in toxicity, as shown by the results obtained with tutocaine (Schulemann, 1924) and larocaine (Frommherz, 1930).

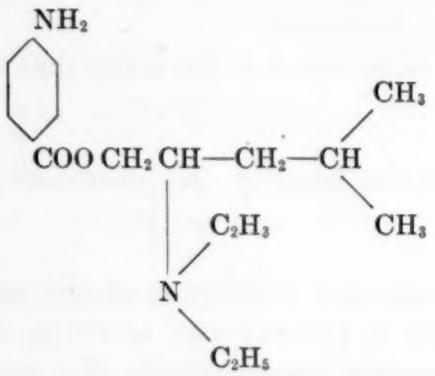


Tutocaine



Larocaine

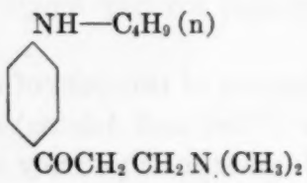
Another substance of the type of tutocaine is panthesine Karrer (1921)



Panthesine

As might be expected from the length of the chain, Röthlin (1929) found that this substance is a good surface and local anesthetic, 2 to 3 times as toxic as procaine, better than tutocaine and psicaine and about as good as cocaine as a surface anesthetic.

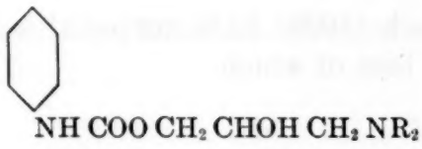
Substitution in the NH_2 group of the aminobenzoic acid radical in this series does not seem to lessen its anesthetic potency as in the case of the insoluble aminobenzoic acid esters (see above) but tends rather to increase it, as shown by the new local anesthetic pantocaine, from two to three times as toxic as cocaine, but good both as a surface anesthetic and for spinal anesthesia. (Fussganger and Schaumann, 1931, and Ernst, 1931.)



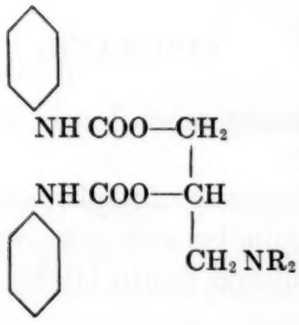
Pantocaine

Other aminobenzoate compounds with somewhat similar side chains have been prepared, as for example the cyclohexanol derivatives of Osterberg and Kendall (1921) but these have not attained widespread use.

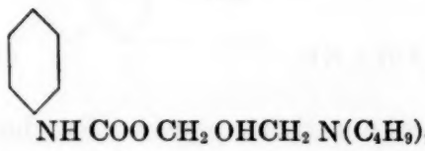
Phenyl urethanes containing the groups C_6H_4 NCOO R and $NH_2C_6H_4COO$ R have been investigated by Frommherz (1914) and by Bonar and Sollmann (1921–22) and found to be of little value but Rider (1930) has prepared two new series of compounds.



and



the best members of which have greater anesthetic power and less toxicity than cocaine. The best of these is



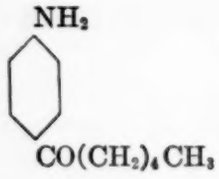
Both the n-butyl and the iso-butyl compounds are six times as strong as cocaine on the rabbit's cornea and less irritating. On the tongue both are stronger than cocaine but the iso-butyl is slightly irritating while the n-butyl is not. They are from three to six times as strong as cocaine in intradermal anesthesia; and they are slightly astringent which seems to prolong their local action. Strangely enough their anesthetic power is increased by boiling. Their toxicity for mice is from one-tenth to one-third that of cocaine.

Derivatives of para phenylendiamine

similar to those of p-aminobenzoic acid are now being prepared by Fourneau and Samdahl (1930) who state that one of them

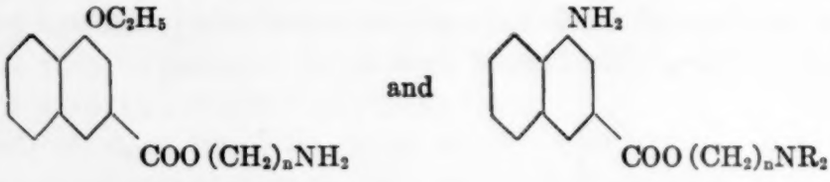
is twenty-two times more potent than cocaine.

Hartung and Munch (1929) have prepared a large series of basic amino phenones, the best of which



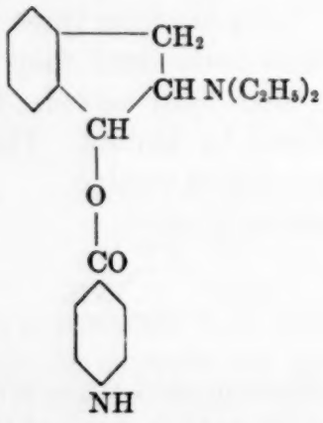
has a low toxicity and causes much longer anesthesia on the rabbit's cornea than does cocaine.

The possibilities of derivatives of naphthalene similar to those of the basic benzoic acid and aminobenzoic acid series seem to have almost escaped attention, but Hill and Smith (1929) have prepared a series of compounds



which have definite local anesthetic power. Further studies along these lines seem promising.

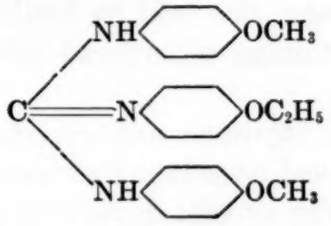
Marvel and du Vigneaud (1924) combined p-aminobenzoic acid with substances having a hydroxindene nucleus, homologues of



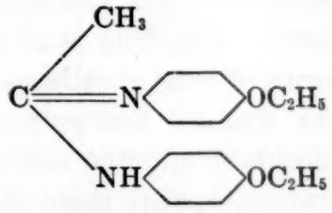
in the hope of obtaining a local anesthetic substance with a vasoconstrictor or pressor action. These substances have some local anesthetic effect but the pressor action was not obtained.

Phenetidin and other similar substances. The antineuralgic effects of phenetidin C_2H_5O⬡NH_2 compounds led to their use in the synthe-

sis of the strongly basic local anesthetics acoine and phenacaine (holocaine, Täuber, 1897).



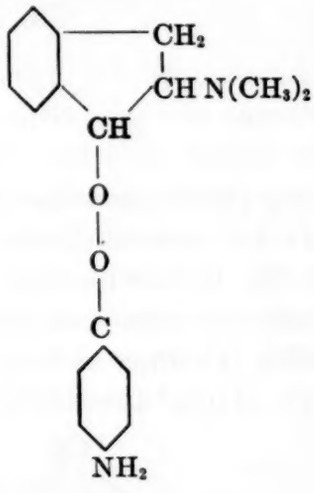
Acoine



Phenacaine (U.S.P.)
(Holocaine)

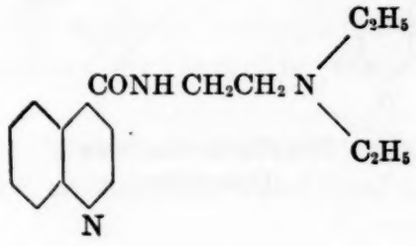
The former has fallen into disuse in recent years, but the latter on account of its high anesthetic power upon the cornea and conjunctiva is still widely used in ophthalmological practice.

Marvel and du Vigneaud (1924) attempted to prepare a series of substances in the hope of obtaining local anesthetics which should also cause vasoconstriction and rise of blood pressure. They, therefore, synthesized



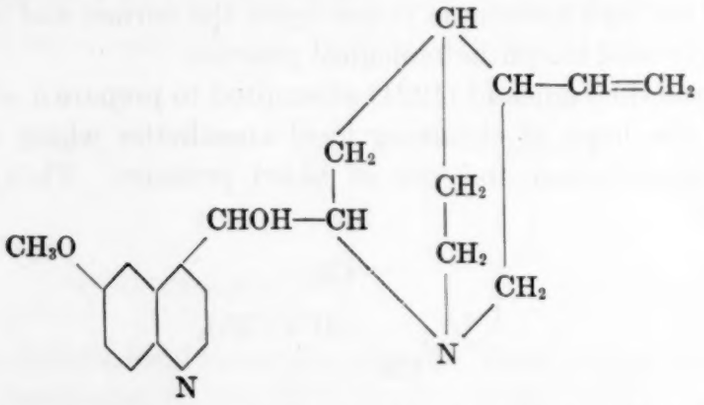
and its ethyl and butyl homologues.

Quinoline anesthetics. Recently these effects have been obtained through synthesis of quinoline compounds, e.g., the potent local anesthetic "percaine" or "nupercaine" (Miescher 1929, Uhlmann 1929, Hassner 1929).

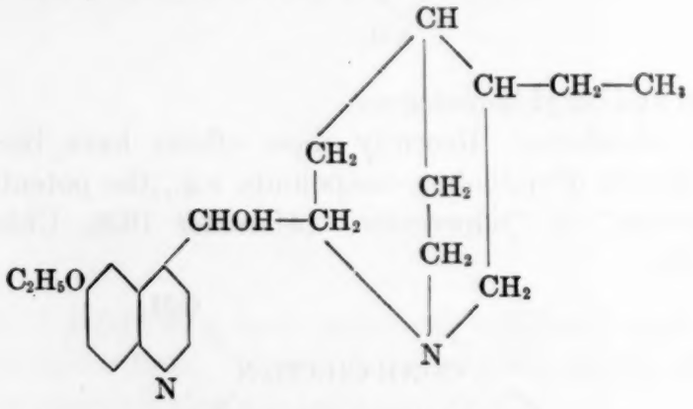


This substance is 10 to 20 times as potent as cocaine but five times more toxic. It produces local vasoconstriction without addition of epinephrine, but this vasoconstriction may also lead to local necrosis (see below). The drug is at present attaining widespread clinical use for all forms of local anesthesia.

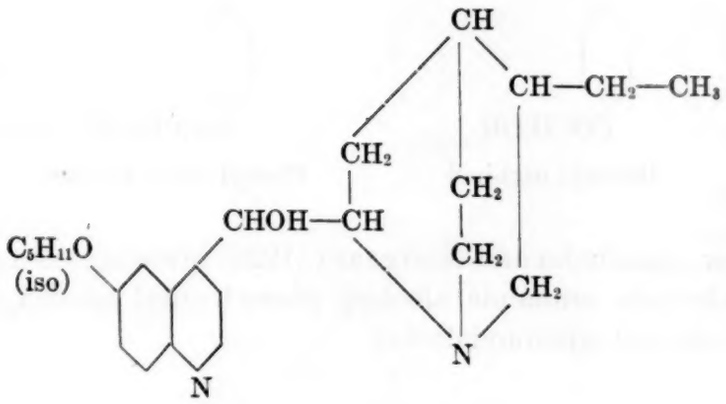
Quinine and the hydrocupreine series. Quinine and especially the hydrochloride of quinine and urea are local anesthetics of particularly long duration, lasting many hours or even several days. On account of their tendency to injure the tissues, however, they are most useful in anesthesia for hemorrhoid operations, where a slight sloughing of tissue does no harm.



Morgenroth and Ginsberg (1912) used the quinine derivative ethyl hydrocupreine (optochine) for subconjunctival anesthesia and later (1913) demonstrated that the hydrocupreines with longer side chains especially the iso amyl hydrocupreine (eucipine) has still longer anesthetic action upon the cornea (25 times as long as cocaine). They also recommend it for other types of local anesthesia.



Ethylhydrocupreine (Optochine)

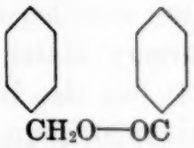


Isoamylhydrocupreine
(Eucipine)

Excellent general discussions of the use of other substances as local anesthetics and of the comparative pharmacology of the rarer substances are given by H. Braun (German editions, not English translation), by S. Fraenkel and by Tiffeneau; while the purely chemical aspects are discussed by E. Waser, Barrowcliff and Carr and Fourneau (see textbooks).

Aromatic alcohols. Macht (1918) demonstrated that the slightly local anesthetic action of papaverine and narcotine are due to the benzyl group which they contain, and that benzyl alcohol itself is a definite local anesthetic. He found that concentrated benzyl alcohol anesthetizes the mucous membrane of the mouth and can be used also to anesthetize dentine in dental practice; and that 1 per cent benzyl alcohol can be used for infiltration anesthesia in tissues. It is, however, only slightly soluble in water and is readily oxidized to benzaldehyde, which is somewhat irritating.

Benzyl alcohol



Benzylbenzoate
(Not anesthetic)

Macht showed that the alcoholic hydroxyl is necessary for the anesthetic action, for benzyl esters (benzoate, etc.) are entirely devoid of it.

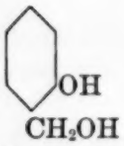
Benzoylcarbinol has a similar local anesthetic action (Hjort and Eagan, 1919) as has phenylethyl alcohol (Hjort and Kaufmann, 1920).

Benzoyl carbinol

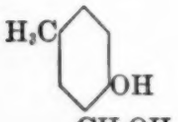
Phenyl ethyl alcohol

Hirschfelder, Lundholm and Norrgaard (1920) investigated a number of aromatic alcohols, cinnamic alcohol, phenyl ethyl alcohol, saligenin, homosaligenin and piperonylalcohol

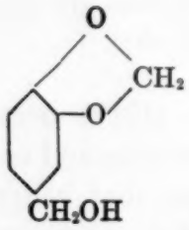
Cinnamic alcohol



Saligenin



Homosaligenin

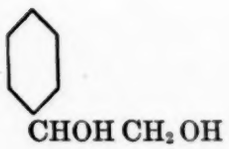


Piperonylalcohol

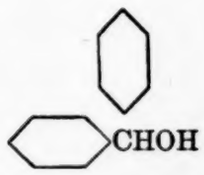
and found that of this series, saligenin is the best local anesthetic. It has no greater toxicity than benzyl alcohol and only one-fifth the toxicity of procaine. While saligenin solutions do not anesthetize the mucous membrane of the mouth, two per cent solutions can be used for infiltration anesthesia, though Farr (1923), who used it in 25 cases of major surgery, states that it is somewhat slower in its action than procaine. It has also been used by Joachimoglu (1925). Hirschfelder and Wynne (1920) found that 4 per cent solutions gave anesthesia in the female urethra satisfactory for cystoscopy, catheterization, etc., and Hirschfelder, Wethall and Thomas (1922) obtained similar results in the male urethra, though they also used 8 per cent solutions of saligenin.

Gardey (1911) found that phenol glycerin ether was only a very weak local anesthetic.

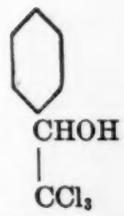
Quigley and Hirschfelder (1921) investigated the local anesthetic action of the secondary aromatic alcohols, phenylglycol, benzhydrol, trichlormethyl phenylcarbinol and cyclohexanol and noted



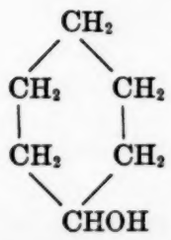
Phenylglycol



Benzhydrol

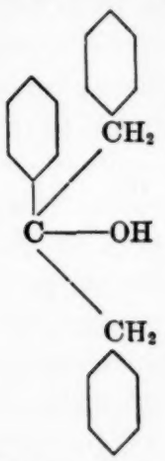


Trichlormethyl-phenylcarbinol

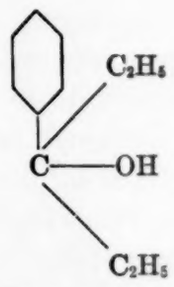


Cyclohexanol

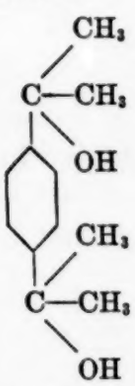
that they are much weaker than the primary aromatic alcohols, and that the tertiary aromatic alcohols, diethyl phenylcarbinol, dibenzyl phenylcarbinol and tetramethyl paraxylenol



Dibenzylphenyl carbinol

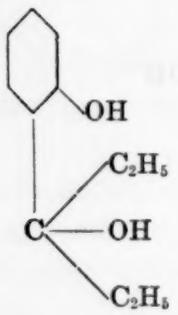


Diethylphenyl carbinol



Tetramethyl paraxylenol

are entirely inert, though di-ethyl-o-oxy-phenylcarbinol



Di-ethyl-o-oxy-phenylcarbinol

on account of its phenolic hydroxyl retains a slight local anesthetic action.

Hirschfelder and Jensen (1921–23) found that several ethers and esters of saligenin though still able to anesthetize the frog's skin and sciatic nerve are too irritant for practical use.

DISCUSSION OF INDIVIDUAL LOCAL ANESTHETICS[5] (SPECIAL CHARACTERISTICS, USES AND TOXIC REACTIONS). *Cocaine.* Despite the fact that the drug is the most dangerous local anesthetic from the standpoint of habituation, and is also one of the most toxic of the group, it is still considered a standard with which new anesthetics are compared. At present, attempts are being made to restrict its use to surface application. It is used in this regard for anesthesia in the eye, nose, throat and urethra, although in the last two named regions it is extremely toxic. Numerous fatalities have been reported from its use, examples of which are: From injections of 0.016, 0.40 and 0.60 gram (Groede, 1912); 0.9 gram from skin infiltration by mistake (Mayer, 1924) and 0.16 gram of powdered cocaine plus epinephrine swabbed on tonsil (Mayer, 1928). According to Sollmann (1930) the ordinary fatal dose is considered to be about 1.2 grams. However, Mayer (1924) and the Committee for the study of toxic effects of Local Anesthetics of the American Medical Association recommended a maximum dose for cocaine of 0.06 to 0.1 gram.

Procaine. This drug is ordinarily considered to be one of the least toxic of the local anesthetics (Mayer, 1928). Up to the present it has been considered the standard for injection anesthesia where it produces a rapid and complete anesthesia that is non-irritant; whereas for surface application it is relatively less active. It should not be used for in-

[5] Many of the toxic reactions are quoted from O. Seifert. Die Nebenwirkungen der modernen Arzneimittel, Leipzig, 1923, 2nd ed., and from Braun, H.; Die oertliche Betäubung, Leipzig, 1921, 6th ed.

jection anesthesia in a concentration greater than 1 per cent. It is, however, capable of producing death in small doses as is evidenced from the following reports; 0.02 gram (Hatcher and Eggleston, 1916); 0.03 gram tonsil injection (Mayer, 1928) and 0.15 gram, tonsil injection (Mayer, 1924). For further discussion of cocaine and procaine see Sollmann's *Manual of Pharmacology*.

Tropacocaine. Tropacocaine introduced by Giesel (1891), much less toxic than cocaine (see table), was the first substitute for cocaine, useful for infiltration anesthesia, nerve block, and spinal anesthesia. In fact until the introduction of procaine for the latter purpose, tropacocaine, for over twenty years, was the drug of choice for spinal anesthesia. It is devoid of the vasoconstrictor action of cocaine (Seifert) and may produce some transient localized edema about the site of injection (Braun). Unlike cocaine it is not injured by sterilization. Its general and toxic effects are otherwise much like those of cocaine.

Psicaine (French "Delcaine"). Synthesized by Willstätter, Wolfes and Maeder (1923) it is d-pseudo cocaine, isomeric with cocaine. The base is more soluble in lipoids than cocaine, and it is more easily destroyed in the body so that less appears in the urine (confirmed by Regnier and Mercier, 1929). Gottlieb (1923) on investigating a series of these cocaine-like substances found that the spatial arrangement in the pseudo form is that most favorable to the development of anesthetic properties. Asal and Lurz (1924) concluded that psicaine is more valuable for surface anesthesia than for infiltration, and found that 1 to 2 per cent or even 0.5 per cent when supplemented with epinephrine is good for cystoscopy and $\frac{1}{4}$ per cent satisfactory for anesthesia of the bladder. This is confirmed by Goldenberg (1926). Kneucker (1927) applies 0.014 gram on a cotton pledget to the mucous membrane of the mouth before puncturing through it with the needle in dental practice. Weiss (1926) used 1 per cent psicaine satisfactorily for removing foreign bodies from the cornea and Rumbaur (1925) finds that it does not injure the latter as does cocaine. Brodt and Kummel (1924) anesthetized the larynx with 1 per cent psicaine. Bettmann (1927) used a 5 per cent solution of psicaine base in lipoiodal for injection into painful or pathological joints. Rumbaur notes the same mydriatic action as with cocaine and states that it is half as toxic as the latter but Kneucker warns that 0.056 gram should be regarded as the maximum safe dose in human beings.

Eucaine A. Vinci and Merling (1896). The first true synthetic substitute for cocaine slightly less toxic than cocaine for animals, and a less

potent anesthetic. Anesthetizes mucous membranes of the mouth and eye but is irritating, especially to the latter. Edema about site of injection has brought it into disuse.

Eucaine B. Vinci (1897). Quite similar in action to eucaine A, slightly less toxic to the tissues but still quite irritant to them, so that the injection of even 1 per cent solutions may cause marked edema of the gums (Turner, 1897; Ornstein, 1897; Wolf, 1913), and general symptoms of flushing of face, nausea and collapse (Braun, Engelmann, 1900; Silbermark, 1904; Lange, 1904; Fink, 1909). Molek (1906) has actually found some toxic phenomena in 20 per cent of the cases in which he used it. Upon the mucous membranes it is equally irritating, producing lachrymation, blepharospasm, hyperemia and alteration of the cornea (Vinci, 1897; Wüstefeld, 1898; Wintersteiner, 1906; Vollert, 1896, Marcinowski, 1902) so that it should never be used in the presence of iritis. In the urethra and in spinal anesthesia it is equally toxic, causing severe symptoms and even death.

Neothesine. Of the large series of benzoyl piperidine derivatives which has been prepared by McElwain and his collaborators, the best is the substance known as neothesine. Coles and Rose (1931) have found that in ordinary use this substance is completely destroyed in the body, but when given in convulsive doses the greater part is excreted within 8 hours. (They also give an excellent bibliography of this phase of the metabolism of local anesthetics.) Meeker (1930) reports satisfactory results using $\frac{1}{4}$ to $\frac{1}{2}$ per cent solutions with and without epinephrin in 60 surgical cases, using amounts as high as 87 mgm. without noting any unpleasant after effects. In intradermal wheals the duration of anesthesia was about 50 per cent longer than with the corresponding concentration of procaine. Walther (1931) used 2 to 4 per cent solutions in the urethra and produces good surface anesthesia for all types of genito-urinary surgery, and his results have been confirmed by Hamer (1931).

Stovaine introduced by Fourneau in 1904 is the only one of the local anesthetics which produces motor block in lower concentrations than sensory (Sollmann, 1918). In line with this observation Braun finds that it is not a very satisfactory local anesthetic and states that 1 per cent solutions cause some stinging on injection and some edema about the wheal.

Alypine. (Impens, 1905). A very similar substance chemically, and it is a much better local anesthetic. In 2 to 5 per cent solutions it produces anesthesia on the mucous membranes of conjunctiva, nose,

larynx and in the urethra. Schroeder (1913) finds its toxicity about half that of cocaine, but it is still to be regarded as a very toxic drug, and has caused numerous toxic effects and fatalities. The dose which is safe for clinical administration is less than 0.2 gram.

Pantocaine. A new local anesthetic has been found by Fussganger and Schaumann (1931) to give superficial anesthesia (rabbit's cornea) in great dilution 1/10,000, i.e., about ten times as powerful as cocaine, with from three to five times the toxicity of the latter. Even in 2 per cent concentration it does not injure the tissues of the cornea. On the frog's sciatic it is as active as procaine in one-tenth the concentration. Ernst (1931) has used it in $\frac{1}{2}\frac{1}{0}$ per cent solutions with satisfaction in 120 major operations, and reports that the anesthesia which is comparable to that obtained with 1 per cent procaine lasts decidedly longer than that obtained with the latter. For spinal anesthesia he injected 1.5 cc. of the 0.5 per cent solution between the third and fourth lumbar vertebrae. Lundy and Essex (1931) (who give a bibliography of 15 titles) have used 1 per cent solutions with satisfaction for surface but prefer it for spinal anesthesia, limiting the amount used to "1 mgm. for each 10 lbs. of body weight, plus 5 mgm." added to the total. In order to prevent fall of blood pressure they give 25 to 50 mgm. ephedrine sulphate intramuscularly to patients undergoing operations on the lower abdomen and 50 mgm. for those with operations at higher levels.

Nupercaine (Percaine) is a methoxyquinoline derivative about five times as toxic as cocaine and about ten times as effective on the mucous membranes. One-twentieth per cent solution will cause anesthesia of the human tongue (c.f. Journ. Amer. Med. Assoc., 1931, xcvi, 943). Its anesthetic action lasts much longer than that of cocaine and procaine (Lipschitz and Laubender, 1929). Unlike other local anesthetics it causes vasoconstriction and in small doses rise of blood pressure (Lipschitz and Laubender, 1929) but on the other hand in toxic doses it depresses the heart more than the respiration. For infiltration anesthesia percaine can be used in $\frac{1}{2}\frac{1}{0}$ to $\frac{1}{1}\frac{1}{0}$ per cent solution, but owing to its very low basicity, upon which the high activity of the substance may depend, the solutions used must be slightly acid (pH 6.2–6.5) and no alkali should be added. Even these may be followed by some edema, ischemia and delay in the healing of the tissues, though usually healing is uneventful. Owing to the high toxicity a number of deaths have been reported. V. Seemann (1929) and Freund (1929) who report one of these think that 0.1 gram should be the maximum dose. On comparison with the effects in animals, even this limit appears to be

too high. At the University of Minnesota Hospital a death followed the injection of 15 cc. 1 per cent solution (0.150 gram) for tonsillectomy.

On the other hand Nupercaine seems to be very useful when applied as in an ointment of one per cent strength for itching burns, pruritus, etc., (Goldhahn, 1930), for in this mode of application, the intense action and long duration of anesthesia come into play, while the high lipoid solubility of the base prevents much absorption.

Butyn (Sulphate) described by Kamm, Adams and Volwiler (1920) and Volwiler (1921) is less basic, more potent and more toxic than procaine, but of about the same toxicity as cocaine, Bonar and Sollmann (1921–22), Nielsen and Higgins (1923). It anesthetizes the tongue and other mucous membranes about in the same degree as does cocaine. It does not have the vasoconstrictor action of cocaine. There is thus far no evidence that butyn is habit-forming. It certainly has a wide sphere of usefulness, provided its toxicity is carefully borne in mind; but the great optimism and lack of precaution originally expressed by Bulson (1922) is quite unwarranted. Thus, while O'Brien (1928) could use 2 per cent butyn solutions for instillation in his opthalmologic practice, Newton (1927) reported a death from instillation of 5 per cent butyn upon the nasal mucosa, and other deaths have been reported especially in cases where the toxicity of the drug has not been duly regarded. With ordinary doses Newton and Lemoine (1927) have found conjunctivitis and dermatitis resulting from the use of butyn. On the other hand, LaRochelle and LaRochelle (1929) have used large amounts (250 cc.) of very dilute 0.1 per cent butyn solutions in surgical practice without ill effects.

Tütocaine was found by Schulemann (1924) to lie between cocaine and procaine in toxicity and speed of elimination. It is an active local anesthetic both for surface application and for injection. Its most useful field appears to be surface anesthesia, which was reported upon satisfactorily by Watson-Williams et al. (1925). Copeland (1924) found that it was relatively non-irritant to the rabbit's cornea and that it was the only other local anesthetic drug which, experimentally, would both anesthetize and shrink the nasal mucous membrane like cocaine. Braun (1924) reported upon its use in major surgery. He says that a $\frac{1}{8}$ per cent solution corresponds to $\frac{1}{2}$ per cent procaine for infiltration. For spinal anesthesia, he recommends the comparatively small dose of 0.6 to 1.0 cc. of $\frac{1}{4}$ per cent solution. Fromherz (1930) compared the bladder absorption of cocaine, procaine, tutocaine, alypine and larocaine and found that the percentage absorption was lowest with tutocaine.

Larocaine was prepared by C. Mannich and reported upon by Fromherz (1930). This anesthetic is somewhat less toxic than cocaine but upon the rabbit's cornea produces an anesthesia in a lower concentration and of much longer duration. It is moreover non-irritant. Compared to procaine it is anesthetic to the sensory nerve fibers in the frog in one-half the concentration of procaine. Koch (1931) has tried tutocaine, psicaine, alypine and nupercaine as substitutes for cocaine in the eye but has rejected all of them. On the other hand he finds larocaine in a 5 per cent solution as satisfactory as 10 per cent cocaine. Its anesthetic action comes on rapidly, it is more powerful than cocaine and he stresses the fact that it is much less irritant than any of the other anesthetics he has tried.

Insoluble esters of para aminobenzoic acid. The insoluble esters of para-aminobenzoic acid, the first of which, ethyl aminobenzoate (anesthesine), was introduced by Einhorn and Heinz (1897–1899), are used clinically by direct insufflation of the powder, after incorporation in ointments, as solutions in oil, as emulsions of these oily solutions, and as simple suspensions or emulsions in acacia for the anesthesia of eroded, ulcerated and itching surfaces; for relieving pains of burns, hemorrhoids, etc. The pharmacology of anesthesine is discussed by Binz and Kobert (1898). Propaesine, cycloform, orthoform much used in Europe and butesine, very popular in America in the form of the picrate, belong to this series. Hotz (1914) found that powdered ethylaminobenzoate (sterilized by recrystallization from alcohol or benzene) could be dusted upon open wounds and caused great relief from pain and no pus formation. Luders (1912) and v. Boltenstern (1912) found that 0.5 gram doses three times a day administered in capsules, were useful in treating vomiting from gastritis, in acute gastric catarrh, gastric ulcer, gastric carcinoma, and seasickness. They also found that sucking lozenges of this substance relieved the symptoms of tonsillitis and pharyngitis. Mixed with milk sugar and applied in the nose they obtained good results in hay fever, coryza, and nasal ulceration. A small amount of ethyl aminobenzoate applied to the wound after the extraction of teeth diminished the after pain. For the treatment of hemorrhoids, pruritus ani, etc., they used 15 per cent ointment. Kaercher (1913) also used it successfully for various forms of vomiting and for the relief of gastric symptoms in both achylia and hyperchlorhydria; and Rosenhaupt (1913) administered it before meals for the nervous vomiting of children and for various forms of hyperirritability of the gastrointestinal tract.

Gast (1920) obtained relief by applying a 5 per cent solution of anesthesine in olive oil to the mouth and tongue in glossitis and pharyngitis.

Hübner (1912) applied a mixture of anesthesine 1, absolute alcohol 10, liquor alumini acetatis 2.0, glycerine 30.0 on sterile gauze strips introduced in the ear down to the inflamed tympanic membrane and claims to have obtained as good relief from pain as from solutions of phenol without its danger of corrosion. Similar beneficial results may be obtained with the other members of the series.

On the other hand, especially after long continued use, symptoms of local and general intoxication may set in; edema, erythema, even erysipelatous eruption in the skin, delayed healing, even localized gangrene (Asam, 1899).

Friedlaender (1900), Wassermann (1902) mention edema of the vocal cords following the use of orthoform on an ulcerated larynx. Albertoni (1898) mentions intoxication from orthoform. Pouchet (1899) warns against applying these substances to the nipple for the relief of pain from erosions, on account of the danger of poisoning the baby.

Braun states that for internal use the maximum single dose should be regarded as 0.5 gram. He found that the soluble salt of ethylaminobenzoate with phenolsulphonic acid was painful on injection, and gave rise to edema of the tissues.

ACTIONS ON CIRCULATION, RESPIRATION, ETC. *Circulation.* Cocaine when injected, into the circulation of any of the experimental animals, in small doses, produces a rise in blood pressure, and in large doses, a drop in blood pressure. Anrep (1880) found that the blood pressure rise produced by small doses was inhibited to a certain extent by section of the splanchnics, whereas the effects of large doses were uninfluenced by splanchnic section. He also observed that small doses markedly increased the pulse rate, whereas large doses decreased it. The marked fall of blood pressure produced by the injections of large doses he concluded was due to depression of the medullary centers and cardiac muscle. Berthold (1885) found that the blood pressure rise was absent if the cord was cut, pointing to a central stimulating effect of cocaine. Mosso (1887) showed that both central stimulation of the cardiac accelerators and vasomotors and peripheral vasoconstriction were factors in the blood pressure rise, but that the central effects were the more prominent. Inhibition of the vagus center is also a factor (Anrep, 1880).

Vinci (1897) found that eucaine B produces a decrease in blood pressure which he attributed to central and peripheral paralysis of the vasomotors; and Marcinowski (1902) confirmed these results, but concluded they were due primarily to peripheral paralysis of the cardiac-accelerator mechanism which resulted in a slowed heart rate.

Impens (1905) observed that alypine in small doses produces a slight increase in the blood pressure due to stimulation of the heart muscle. With larger doses the blood pressure falls due to central and peripheral vasomotor paralysis and to depression of the heart. The decrease in blood pressure, however, is not as marked as that obtained with cocaine. The effects of procaine on the blood pressure are quite similar to those produced by cocaine, but they are less marked (Roth, 1917). Stovaine, on the other hand (Smith and Hatcher, 1916–17), has only a depressive effect on the heart, which results in a decrease in blood pressure.

Butyn (Nielsen and Higgins, 1923) in small doses first produces slight stimulation of blood pressure, then a short decrease followed by a prompt return to normal. With larger doses the decrease in blood pressure is more pronounced and the return to normal much slower. They also observed that the fall of blood pressure and convulsions, in dogs, could be prevented by the injection of pituitary solution. Schulemann (1924) observed that tutocaine was very similar to procaine in its effect on blood pressure, except that it had a more powerful effect. Copeland (1924) studied the effects of 5 mgm. doses of a number of local anesthetics on blood pressure. He noted that cocaine produced a marked rise of blood pressure, tutocaine, a smaller rise of blood pressure, and butyn, a decrease in blood pressure, with gradual recovery. Panthesine (Rothlin, 1929) also produces first a rise of blood pressure followed by a marked decrease. This drug appears to be about twice as powerful as procaine in producing these effects.

Nupercaine (Uhlmann, 1929) in very small doses produces either no effect or a slight drop in blood pressure, whereas in large doses it produces a powerful drop in blood pressure. Lipschitz and Laubender (1929) obtained the same results. Moreover, they found that in rabbits the heart was more sensitive to nupercaine than the respiratory center and that cardiac depression was the more common cause of death. Larocaine (Fromherz, 1930) appears to produce only a decrease in blood pressure. Pantocaine (Fussganger and Schaumann, 1931) also produces only a decrease in blood pressure. Lundy and Essex (1931) obtained the same results, but add that, considering its toxicity, relatively large doses are necessary to produce this drop in blood pressure. Wiedhopf (1931) compared the effects produced by a number of new local anesthetics in dogs. Pantocaine, 4 mgm. per kgm. produced a drop in blood pressure from 110 to 70 mm. Hg for 40 seconds, which became normal in $3\frac{1}{2}$ minutes. Nupercaine, using the same dose, produced a drop in blood pressure from 70 to 20 mm. Hg followed by a rapid return to

normal. This dose, however, produced cardiac irregularities and convulsions. Tutocaine, ten times the above dose, caused a drop in blood pressure from 80 to 20 mm. Hg. This dose also injured the heart and stopped respiration. Artificial respiration resulted in recovery and return of the blood pressure to 54 mm. Hg seven and one-half minutes later.

Respiration. Cocaine (Anrep, 1880) in small doses stimulates respiration in laboratory animals. With larger doses, the period of stimulation is short and is followed by a depression which progresses to standstill as the cause of death. Mosso (1887) showed that the increase in rate and depth of respiration was due to central stimulation and that death was due to paralysis of the center. Roth (1917) found that death from cocaine was always respiratory. He also found that procaine produced only a slight increase in respiration, as compared to cocaine, which was followed by a depression. The cause of death was generally respiratory, but occasionally cardiac.

Eucaine B (Vinci, 1897) in small does and early in a toxic picture stimulates respiration. Later, with larger doses, respiration becomes shallow and slow, during convulsions it is irregular and dyspneic. Death is due to paralysis of respiration. Marcinowski (1902) obtained the same findings.

Impens (1905) studying alypine found that small doses decrease the respiratory rate but increased their depth. Larger doses increase both the rate and depth of respiration, which finally becomes depressed and then stops before cessation of the heart.

Stovaine (Smith and Hatcher, 1916–17) produces first respiratory stimulation which later gives way to depression. They give as the cause of death, simultaneous paralysis of the respiratory center and heart. Butyn (Nielsen and Higgins, 1923) appears to be somewhat less depressing on the respiratory center than cocaine; moreover, because, experimentally, these investigators were able to prevent fatality in dogs with pituitary liquid, one might suspect that butyn is more toxic on the circulation than on respiration. Tutocaine (Watson-Williams, 1925) depresses respiration which progresses to paralysis as the cause of death. Panthesine (Rothlin, 1929) in small doses produces some respiratory stimulation; in larger doses, respiratory depression, with irregularities. These effects, very like those produced by procaine, occur with about one-half the dose of procaine necessary for the same degree of action. Schmitz and Loevenhart (1924) report that cocaine, procaine, isocaine, and butyn all produced respiratory stimula-

tion in the intact animal as one of the first symptoms observed, and that in all cases respiratory paralysis was the cause of death.

Nupercaine (Uhlmann, 1929) first accelerates respiration then produces depression. Paralysis of respiration occurs rather late. Larocaine (Fromherz, 1930) produces respiratory depression which progresses to respiratory failure as the cause of death. Fussganger and Schaumann (1931) have found that respiratory paralysis is the cause of death following the administration of pantocaine. Lundy and Essex (1931) have confirmed this, and have observed in addition that non-fatal doses cause stimulation of respiration.

Convulsions. Cocaine and the other local anesthetics produce convulsions as another result of their stimulant effect upon the central nervous system. Feinberg and Blumenthal (1887) found that in dogs, cocaine convulsions appear to be confined to the cerebral cortex. Morita (1915) found in rabbits with the cerebral hemispheres removed that clonic convulsions and respiratory effects were absent, but that tonic convulsions and "running movements" were present. Mosso (1887) observed that, during the convulsions in dogs, the blood pressure increased and that respiration became irregular and deep. He concluded moreover that the convulsions were not reflex as with strychnine. According to Sollmann (1930) the convulsions are probably descending, and different convulsive centers may be affected in succession. Poulsson (1920) says that the motor cortex shows increased sensitivity to electrical stimulation along with the convulsive movements and respiratory and circulatory changes. Furthermore the period of stimulation merges with one of depression especially with larger doses. In cold blooded animals the period of stimulation is fleeting or absent, while the period of depression is marked. Among mammals, the higher the animal is in the developmental scale, the greater the depression. Again, Dixon (1912) has shown that the greater the brain development the smaller the dose of cocaine necessary to produce convulsions, it requiring approximately $\frac{1}{15}$ as much cocaine to produce convulsions in the ape as in the rabbit. The picture in man is described by Sollmann (1930) and by Lodge (1912).

Tonic and clonic convulsions are described by Vinci (1897) due to eucaine B. They finally give way to a state of depression. Marcinowski (1902) found that the convulsions produced by eucaine B are not as powerful as those produced by eucaine A. Rather, the toxic picture of eucaine B is one of marked paralysis of the peripheral motor apparatus simulating the effects of curare.

Alypine, on the other hand (Impens, 1905), is a markedly convulsive drug. In the dog there is first what appears to be psychic stimulation followed by tonic and clonic convulsions. The picture is so marked that Impens ascribes the cause of death from alypine to exhaustion. In the cat and rabbit there is less psychic stimulation, but the convulsions are just as marked. Further support as to the mechanism of death he adduces from the fact that the hypnotic "Isopral" (trichlor-isopropyl alcohol) will protect the cat and rabbit from the alypine convulsions and then these animals can tolerate more than the ordinary fatal dose. The respiratory stimulation from alypine is not destroyed by isopral. He also found that isopral would not protect his animals from cocaine.

Heinecke and Laewen (1905) obtained convulsions in experimental animals with procaine. With large doses there is rapid paralysis of the extremities, the animals fall over on their sides, show respiratory changes and then develop clonic convulsive movements of the extremities (Laufkrampfen, Biberfeld) and also tonic convulsions. Later this gives way to depression. Convulsive and depressive states are described for isocaine, butyn, procaine and cocaine by Schmitz and Loevenhart (1924). Tutocaine convulsions were noted by Schulemann (1924) and Watson-Williams (1925). Schulemann found that procaine produced strong convulsions and less central depression, whereas tutocaine produced slight central stimulation and strong central depression. Gottlieb (1923) observed convulsions following psicaine. Uhlmann (1929) described convulsions followed by depression, produced by nupercaine. Wiedhopf (1931) found that nupercaine was more stimulant to the central nervous system than either pantocaine or tutocaine. Convulsions due to pantocaine were noted by Fussganger and Schaumann (1931) and by Essex and Lundy (1931). The last named investigators found that the convulsions were similar to those produced by procaine.

Eggleston and Hatcher (1919) studied the toxicity of the following local anesthetics: alypine, apothesine, beta eucaine, cocaine, holocaine, nirvanine, procaine, stovaine, and tropacocaine in cats. The symptoms produced were essentially the same for all of the drugs. Following intravenous administration the symptoms began within a few seconds and ran their course as follows: Pronounced respiratory stimulation; abrupt fall of blood pressure with a slow heart usually, due to weakening of the heart muscle; paralysis of respiration for a few seconds; or continued stimulation; cardiac improvement with a rise of blood pressure occurring within about one minute after the injection; decrease of the

respiratory acceleration; several brief but severe clonic convulsions; and return to normal at about 5 minutes. With nearly fatal doses the return to normal required a longer time and there were more convulsions. Based on time of recovery the drugs could be divided into two groups, one group containing cocaine and holocaine, from which the animals recovered slowly, and a second group containing the remaining anesthetic drugs from which recovery occurred more rapidly.

Supposed effects of local anesthetics upon inflammatory edema. Spiess (1906), Ninian Bruce (1910–1913), and Bardy (1915) were able to inhibit the onset of edema of the rabbit's conjunctiva after the instillation of mustard oil by preliminary anesthesia produced with cocaine or alypine. Ninian Bruce regarded this as proof of the existence of the so-called axone reflexes postulated by Langley (1894–1923), who believed that the sensory fiber was divided into a sort of γ one branch of which terminated in a sensory ending and one in a vasodilator ending. Hirschfelder (1924) however, repeated these experiments using a larger series of anesthetics (cocaine, alypine, butyn, procaine, and saligenin) and found that though cocaine and alypine which themselves cause local vasoconstriction sometimes produce the effects described by the earlier observers, butyn, procaine and saligenin which do not cause vasoconstriction never suppressed the onset of conjunctival edema from mustard oil. Hirschfelder was able to suppress this temporarily by adding epinephrine to these solutions so as to induce vasoconstriction. He also showed that when trypanblue was injected intravenously the area to which the mustard oil was applied took on a much deeper blue color than the normal conjunctiva, showing that the tissue and capillary walls were injured even though no edema was manifest at the time. In all his experiments edema developed in the area subjected to mustard oil as soon as local edema passed off. The amount of edema fluid was roughly proportional to the filtration pressure in the arterioles, was increased by high filtration pressure and decreased by low filtration pressure regardless of whether this was produced by changes in the local or in the general blood pressure. These findings have been confirmed from a different angle by Hanzlik. It would, therefore, appear that local anesthesia per se has no effect upon the genesis of edema or inflammation reactions as postulated by Spiess and Ninian Bruce.

TOXICITY, PREMEDICATION AND ANTIDOTES. Eggleston and Hatcher (1919) have shown that the toxicity of local anesthetics for the cat, and this presumably holds for man, depends upon the ratio between the rates of absorption and elimination.

Absorption from subcutaneous injection can be slowed (except tropacocaine) as is commonly known, by the addition of epinephrine in a concentration not over 1:50,000 (H. Braun, 1903; Sollmann, 1918; Eggleston and Hatcher, 1919). This means that for an anesthesia of a given time a smaller quantity of the anesthetic drug can be employed. That there is danger, locally, in using too great a concentration of epinephrine has been shown by Serafin (1928), who described local slough of tissue from the accidental use of a local anesthetic solution containing epinephrine in a concentration of about 1:20,000. Kohlhardt (1901) has also shown that from extremities, the absorption of a local anesthetic can be slowed by means of a tourniquet applied above the site of injection. To guard against systemic tonic reactions from epinephrine, especially in hypersensitive patients, and from potentiation of the toxic reactions of the local anesthetic, not more than 1 mgm. of epinephrine in a local anesthetic solution should be injected into a patient at any one time (Mayer, 1924). It is important to remember that with varying the absorption of a local anesthetic, that is, the speed with which the drug gets into the blood stream, Hatcher and Eggleston (1916) have shown that an animal will succumb to a smaller dose of procaine (given by rapid intravenous injection) than of cocaine (given by slow intravenous injection).

From the standpoint of elimination, Eggleston and Hatcher (1919) have shown that the then commonly known local anesthetics could be divided into two groups: Group 1, cocaine and holocaine, which are not destroyed or eliminated rapidly by the organism; and group 2, alypine, apothesine, B-eucaine, nirvanine, procaine, stovaine, tropacocaine, which are rapidly destroyed or eliminated by the organism. This rapid destruction and elimination occurs chiefly in the liver, where it can be demonstrated by perfusion experiments. These investigators found, in the cat, that a fatal dose of any of the local anesthetics in group 2 was destroyed in about twenty minutes, whereas in group 1, a fatal dose of holocaine required about thirty minutes for destruction and elimination, and a fatal dose of cocaine required not less than sixty minutes.

With the very wide modern usage of local anesthesia, it is surprising that more attention has not been given to the rôle of the liver in the occurrence of toxic reactions. Ellinger and Hof (1929), however, have shown that liver damage, chloroform anesthesia and phosphorus poisoning increase the sensitivity of animals to cocaine, novocaine and tutocaine, and from this conclude, perhaps correctly, that liver damage is a contraindication to the wide use of local anesthesia.

The ratios of absorption and elimination of the local anesthetics, recently introduced, have not been as completely studied as those of the anesthetics in use for over ten years. It has been found that the addition of epinephrine will prolong the anesthesia, and also presumably, decrease the absorption from subcutaneous injection, of the newer local anesthetics, butyn, psicaine, tutocaine, panthesine, neothesine, nupercaine, pantocaine and larocaine. This, therefore, is a factor in decreasing possible toxic reactions.

Some evidence on the speed of destruction and elimination of these newer local anesthetics is also available. Gottlieb (1923) has shown that psicaine is tolerated in cats in more fractional doses than is cocaine, so is probably more rapidly eliminated. Schulemann (1924) found that tutocaine is rapidly eliminated from the organism, and thus appears to behave more like procaine than like cocaine. Nupercaine elimination (Lipschitz and Laubender, 1929) appears to be slow, therefore more like cocaine. Pantocaine (Fussganger and Schaumann, 1931) appears to lie between cocaine and procaine in its speed of elimination from the organism. Larocaine (Fromherz, 1930) is eliminated just as rapidly as tutocaine, with which it was compared. Coles and Rose (1931) studied the elimination of neothesine in dogs. They found relatively little unchanged neothesine in the urine, especially following small doses. Moreover, the major portion appears to be excreted within eight hours after administration. This would indicate that this drug is destroyed and eliminated more rapidly than cocaine (Rifatwachdani, 1913; 42 to 85 per cent excreted in the urine of rabbits; Biehler and Gruhn, 5 to 20 per cent excreted in the urine of cats).

For further prevention of toxic reactions from local anesthetics one should follow the rules laid down by the Committee for Study of Toxic Effects of Local Anesthetics of the American Medical Association (Mayer, 1924). Briefly their recommendations are as follows: Cocaine should not be used for injection, but should be used only for surface anesthesia in a concentration not greater than 5 to 10 per cent, with a maximum dose of from 0.06 to 0.1 gram; for injection anesthesia procaine should not be used in a concentration greater than 1 per cent; if apothesine is used for injection, the maximum concentration should be 2 per cent and the maximum dose 0.1 gram; butyn should not be used for injection anesthesia, the maximum dose to be used should not exceed 0.06 gram. Because accidents due to absorption from the urethra are fairly common, the Committee advises that local anesthesia should not be employed in the presence of trauma or stricture.

Premedication and antidotes. Since in death from poisoning by cocaine and by most of the other local anesthetics the respiratory center fails before the heart, artificial respiration has been a routine procedure in treatment. Eggleston and Hatcher (1919) and Tatum, Atkinson and Collins (1925) have been able by this procedure alone to save the lives of animals given from two to three times the lethal dose.

The administration of respiratory stimulants, such as caffeine, strychnine and atropine have long been recommended as antidotes. Dragstadt and Lang (1928) claim that 1 mgm. atropine sulphate is the best of these drugs and that it would protect rabbits from a lethal dose of cocaine, but Knoefel, Herwick and Loevenhart (1930) have failed to confirm their findings. Even so intense a respiratory stimulant as a minute dose of KCN which markedly increases the action of the respiratory center of normal rabbits had little or no effect after the depression produced by cocaine.

A much more effective method for both prophylactic premedication and immediate treatment of intoxication has been found in the use of the narcotic and soporific drugs. Mosso (1890) noted the fact that the administration of chloral hydrate lessened the sensitiveness of rabbits to cocaine; and Herzfeld (1913) recommended the administration of 25 to 50 cc. of whiskey to patients before the local anesthetic as a prophylactic. He stated that in four years of dental practice he never had a toxic reaction. Hofvendahl (1922) and La Mendola (1924) confirmed the findings of Mosso; but it remained for Tatum, Atkinson and Collins (1925) to furnish conclusive proof that administration of the soporific drugs and especially those of the barbital series, protects against the onset or even stops the convulsive seizures from cocaine and other local anesthetics and raises the lethal dose to double or treble that for untreated animals, and that this is directly applicable in clinical practice. Tatum and Collins (1926) showed that the higher the brain development, as in the monkey, the greater the protection. Knoefel, Herwick and Loevenhart (1930) have entirely confirmed these observations and have demonstrated that the degree of protection is directly proportional to the degree of depression produced, the efficacy increasing in the order chloral hydrate < paraldehyde < sodium barbital < sodium phenobarbital < sodium amytal. They found that urethane and ether have no consistent effect; though Farr (1923) recommends the use of ether as an antidote to procaine poisoning in clinical cases. They found that the alkalinity of the barbitals played no rôle in this protection, though Salant and Nadler (1927) had found that the mere

injection of alkali diminished the sensitiveness of animals to cocaine. Guttman (1928), Leshure (1927), Williams (1927) Barlow (1929) and de Takatz (1930–1931) state that in clinical use premedication with barbitals as recommended by Tatum and his collaborators, greatly reduces the incidence of toxic symptoms. de Takatz (1931) who gives an excellent review of the literature also recommends a just soporific dose of tribromethanol (60 mgm. per kilo, rectally) for the same purpose. It is probable also that the preliminary administration of scopolamine and morphine which Farr (1923) used almost routinely to quiet his patients has a similar protective effect, and that this accounts for the relative freedom of his patients from toxic symptoms in spite of the large doses of procaine and other local anesthetics, which he used routinely.

For failure of the circulation or depression of blood pressure various drugs have been employed.

Lhotak von Lhota (1909) claimed that strophanthine afforded some protection against cocaine. This has been confirmed by Eggleston and Hatcher (1919). Weiss (1923) found no beneficial effect from calcium salts. Eggleston and Hatcher showed that injection of epinephrine exerts a small degree of protective action. In clinical practice the subcutaneous injection of 1 mgm. epinephrine or 25 to 50 mgm. ephedrine is generally resorted to when depression of the circulation sets in, and many surgeons inject this dose of ephedrine before the local anesthetic as a prophylactic measure.

SPECIAL METHODS OF CLINICAL ADMINISTRATION. Local anesthetics are administered in general surgery by either of the following routes: (1) Infiltration anesthesia, surrounding the field of operation completely with a ring of anesthetic diffusely injected into the tissues. This may be used either (a) by injection into the subcutaneous and deep tissues in the vicinity of the incision or (b) as a "ring block" injected intracutaneously or subcutaneously at a distance above the area of operation, so as to block all the nerves before they reach the area of operation, leaving this area itself free from anesthetic. (2) Nerve block, injecting the anesthetic only into the vicinity of the nerve-trunks supplying the area. This also includes the special field of paravertebral and parasacral anesthesia introduced by Sellheim (1905), in which the drug is injected into the vicinity of the dorsal nerve root just as it enters the spinal canal in the intervertebral space. (3) Sacral anesthesia. (4) Spinal anesthesia.

Infiltration anesthesia. Infiltration anesthesia, the oldest and simplest

method can be performed with any local anesthetic in the lowest effective concentrations, one-half to one per cent procaine or equivalent solutions. Halsted and later Schleich showed that the pressure of the injected solution was a large factor in promoting the anesthesia (see p. 191).

The special applications in practical surgery are fully discussed by by R. E. Farr (1923), H. Braun, and the other text-books of local anesthesia.

In contrast to the other methods of local anesthesia for general surgery, Farr claims that "Direct infiltration possesses the obvious advantages of speed, simplicity, wide application, and the important attribute that the technic may be acquired and accomplished with comparative ease,"—while the other methods require far greater skill, knowledge and care.

One of the most interesting applications of infiltration anesthesia is for the reduction of fractures and dislocations after first infiltrating a complete ring to involve all the nerves above the lesion. This was first performed by Conway (1885), and recently has been well described by Rice (1928), Myra and Willis (1930). Braun (1925) gives an account of fractures and 51 cases of reduced luxations of joints in which he produced anesthesia by the injection of one per cent procaine into both the peripheral and the central ends of the joints, and in fractures into areas distal and proximal to the fracture. The injected dose should be carefully observed in certain regions of the body, as for example, in the head and neck region, where according to Eidens (1923) the balance between the vagus and sympathetic system may be easily upset.

Nerve block. For producing nerve block 2 to 3 cc. of the solution (1 per cent procaine or its equivalent) are injected as nearly as possible into the trunks of the principal nerves supplying the area of operation, and also into those of the neighboring nerves whose distribution may overlap. The chief essential is a knowledge of the site of the nerve trunks and of the overlapping nerve supply to the area. The details of this technique as applied to many regions are well given by Pitkin (1930) in an excellently illustrated article.

Henline (1930) and Bumpus (1931) give good descriptions of the technique of regional anesthesia in genito-urinary surgery. The special aspects of nerve blocking are well treated in most of the text-books of local anesthesia and dental anesthesia.

Paravertebral blocking, especially of the spinal nerves, introduced by Sellheim in 1905 requires a higher degree of skill and practice. The

technique is well described by G. Labat (1928) from whose text-book on *Regional Anesthesia* the following procedure is summarized: Labat makes a small intradermal wheal 4–5 cm. from the midline at the level of the corresponding vertebral spine, introduces a long needle through this wheal inclined at an angle of 45 toward the midline and advancing it downward, inward and slightly forward, so that its path crosses the intercostal space. In so doing the needle is moved to and fro while continuously injecting the anesthetic which thus comes into contact with both the anterior and posterior divisions of the nerve and the ramus communicans. From 6 to 10 cc. of $\frac{1}{2}$ to 2 per cent procaine are injected. If the needle impinges on a rib or seems to enter the pleural cavity it is slightly withdrawn and inclined still more toward the midline. Paravertebral anesthesia requires a high degree of skill, especially in the upper thoracic region, where one can easily penetrate into the subarachnoid space or at least the subarachnoid cul de sac which accompanies the nerve beyond the intervertebral foramen. This can be avoided by removing the barrel of the syringe from the needle during the last part of its introduction, and waiting for the appearance of cerebrospinal fluid or even aspirating for the latter. If care is taken to pass the needle between the transverse processes, the danger of producing an epidermal hematoma is minimized. Injection into the pleural cavity usually produces coughing.

As can be seen from the above mentioned complications the procedure is by no means simple or free from risk, though in the hands of skilled operators the results are usually satisfactory.

In the last few years the diagnosis of angina pectoris has been facilitated and the symptoms have been relieved by paravertebral injections of procaine solutions mixed with or followed by 80 per cent alcohol from the eighth cervical to the seventh thoracic nerves. Brunn and Mandl (1924), Swethow (1926–30) and White (1930) have used this method in other regions for the relief of other visceral pains. Swethow showed that the injection of alcohol into the dorsal root gives rise to a definite Wallerian degeneration of the nerve.

Transsacral, parasacral and presacral block. One of the most useful variations of paravertebral blocking is the anesthesia of the branches of the entire sacral plexus by local injections. Labat prefers to inject each sacral foramen separately by injecting 1 per cent procaine solution from the back directly into each sacral foramen through a small anesthetized wheal; using 6 cc. of anesthetic for the first sacral foramen, 5 cc. for the second, 4 cc. for the third, 3 cc. for the fourth, 2 cc. for the

fifth foramen. The solution thus remains in the extradural space and anesthetizes both the anterior and posterior branches. Since it does not enter the spinal canal it is free from most of the dangers and toxic phenomena attending spinal anesthesia and gives satisfactory anesthesia for the corresponding pelvic viscera, though it may have to be supplemented by a certain amount of nerve block or infiltration anesthesia.

In presacral (sometimes termed parasacral block) long needles are introduced along each lateral margin of the coccyx with the patient in the lithotomy position, and passed upward into the hollow of the sacrum where about 100 cc. 1 per cent procaine are gradually injected so as to come into contact with each of the nerves as they emerge from the foramina. This method is said to be very useful for vaginal and genito-urinary surgery, especially when supplemented by additional infiltration or nerve block. Burghardt (1929) fills the presacral cavity with 0.5 per cent procaine, in 0.4 per cent K_2SO_4 for vaginal surgery, using as much as 250 cc. of the solution. Henline (1930) and Bumpus (1931) use it as part of the procedure for regional genito-urinary anesthesia.

Anesthesia of the bladder and urethra. Cocaine, despite its toxicity, still remains the drug of choice for producing satisfactory anesthesia of the bladder and urethra. Procaine, however, is more widely used but is reported to give only an incomplete anesthesia (Randall, 1923, and Hager and Loevenhart, 1930). The reason for this choice is the fear of toxic reactions from cocaine. Most urologists, however, are familiar with both drugs, so that the effects of any new local anesthetics are described in terms of the activity of procaine and cocaine.

Anesthesia of the urethra is produced by either of two general methods: 1, by instillation of a solution of the local anesthetic into the urethra anesthetizing either the anterior or posterior urethra or both, or by applying the solution by means of a swab, and 2, by means of a tablet depositor, placing a tablet of the local anesthetic in the posterior urethra which then goes into solution in what urine is present at this point.

At the Mayo Clinic (Braasch, 1931), $4\frac{1}{2}$ grains of cocaine are dissolved in 1 ounce of fresh sterile tap water and instilled into the urethra immediately after preparation. No unnecessary force or pressure is used in making the injection. The use of forcible injection is to be strictly avoided. In addition $2\frac{1}{2}$ grains more of cocaine in 1 ounce of water are injected into the bladder if necessary. This anesthesia is used for cystoscopy. When dilatation of the urethra is to be employed and

where instrumentation has been recently employed, cocaine is not used for fear of increased absorption from the denuded and abraded mucous membrane. Instead, six tablets of procaine, each containing 0.125 grain, are dissolved in 1 ounce of tap water and instilled into the urethra. These anesthetic methods have been used in approximately 75,000 cases without a death. A number of reactions have occurred, but they were slight and transient. In most cases these reactions occurred in patients who had been dilated or subjected to other trauma several days before. Braasch believes that the absence of toxic reactions in this large series of cases, though fatalities have occurred in other clinics, is due to the above-mentioned precautions.

Blum and Glingar (1924) use 100 cc. of 1 per cent tutocaine for bladder anesthesia, and 2 to 4 per cent tutocaine in water or glycerine for urethral anesthesia. Allowing 5 minutes for the anesthesia to develop they say that 2 per cent tutocaine in the anterior urethra produces as satisfactory an anesthesia as alypine and that it is much safer. It is moreover much more efficient than procaine. Chetwood and Cooney (1926) use up to $\frac{1}{2}$ ounce of 1 per cent tutocaine instilled into the urethra. Kingston (1925) reports that 10 to 20 minims of 1 per cent tutocaine instilled into the urethra gives a satisfactory anesthesia for instrumentation which comes on in three minutes and lasts fifteen minutes. A. G. Wethall uses 2 per cent tutocaine, instilling 10 cc. Waiting five minutes for anesthesia to develop, he reports a satisfactory anesthesia quite comparable to that produced by cocaine.

Fiedler (1928) claims to find procaine borate a better urethral anesthetic than tutocaine. That this is not a general observation is well shown by a patient in Wethall's clinc at the University of Minnesota, who had borne the passage of a sound under tutocaine anesthesia without pain. When procaine borate was substituted, he complained of pain and said, "I guess the doctor left out the medicine." Tutocaine borate, which we prepared, did not seem to have any greater potency than tutocaine itself.

Saligenin (Hirschfelder, Wethall and Thomas, 1922; and Hirschfelder and Wynne, 1920) also gives a fair degree of urethral anesthesia.

G. J. Thomas who formerly used one, and occasionally two, $1\frac{1}{8}$ grain tablets of cocaine introduced into the posterior urethra with a tablet depositor, now is using 2 per cent neothesine. One to two ounces are instilled into the urethra and bladder. Allowing the solution to remain in contact with the tissues for 10 minutes, (by means of a meatus clamp) gives, in his hands, an anesthesia entirely comparable to that produced

by cocaine. In the female urethra he uses 4 per cent neothesine on a dry swab for topical application. Used as above in 250 cases, he reports no toxic reactions. Previously, using cocaine only in patients who appeared to be safe risks, he has had only an occasional non-fatal toxic reaction. His patients are always studied for contraindications to the use of urethral and bladder anesthetics. Walther (1931) uses 4 to 8 cc. of 2 to 4 per cent neothesine instilled into the anterior urethra, followed in a few minutes by 8 to 16 cc. more to reach the posterior urethra. This is allowed to remain in contact with the tissues for from 3 to 10 minutes by means of a meatus clamp. In the female urethra he instills 15 cc. of 2 to 4 per cent into the bladder and then places a swab of the anesthetic solution in the urethra. This has given a satisfactory anesthesia in 1500 cases.

Walker (1930) uses nupercaine 1:750 in the urethra and bladder, but thinks it is not as satisfactory as cocaine, but better than 3 per cent procaine. Keyes and McLellan (1931), (who cover the literature quite thoroughly for nupercaine), use up to 18 cc. of a 1:250 solution (up to 72 mgm.) in the urethra and bladder and report a satisfactory anesthesia in ten minutes. F. E. B. Foley is using 10 mgm. nupercaine tablets injected into the posterior urethra with a tablet depositor, or up to 60 or 70 mgm. in a solution instilled into the urethra. He reports just as complete an anesthesia, allowing a ten minute interval for the drug to take effect, as with cocaine, allowing 2 to 3 minutes for the drug to take effect; and the anesthesia is moreover much more prolonged with nupercaine.

Hager and Loevenhart (1930) report upon the use of isocaine. Sodium bicarbonate is added until the isocaine solution is neutral or slightly basic. To the meatus 10 per cent isocaine is applied on a cotton pledget, then 4 cc. of 10 per cent isocaine is instilled into the posterior urethra through a catheter. Allowing 10 minutes for the anesthesia to develop, this gave a satisfactory anesthesia in 91 out of 93 patients which lasted through the time of instrumentation (up to 20 minutes).

Macht (1918–21) has shown that cocaine, alypine and stovaine are absorbed by the mucous membrane of the urethra, ureters and vagina, and can exert typical toxic reactions upon respiration and blood pressure. He also found that absorption from the bladder was much slower. Sollmann (1918) and Saito (1924) determined quantitatively the rate and amount of this absorption. On this account it is not surprising that a considerable number of intoxications and deaths have been reported from urethral anesthesia, Randall (1923) cocaine 8 deaths and

25 intoxications, alypine 1 death and 3 intoxications, procaine 9 intoxications and apothesine 1 intoxication; Proskauer (1913) death from 0.4 gram alypine; and Jameson (1929) 2 deaths from 0.3 and 0.4 gram butyn in 2 per cent solution.

Bonneau (1907) obviated a great part of this difficulty by the use of cocaine base dissolved in olive oil, which he claimed gave prolonged anesthesia of the urethra. Since the anesthetic bases remain in solution in the oil, and yet enough diffuses out to anesthetize the mucous membrane, it seems probable that this mode of administration is worthy of more extensive trial with other drugs.

On the other hand, it must be frankly admitted that the ideal local anesthetic for anesthesia of the urethra still remains to be discovered.

Spinal anesthesia. Spinal anesthesia was first performed by J. L. Corning in 1885 on a dog. After the successful performance of this he injected three minims of a three per cent cocaine solution between the eleventh and twelfth dorsal vertebrae of a man, upon whom he passed a urethral sound without pain and without loss of motion of the limbs. The method was reintroduced for surgical anesthesia by A. Bier (1889) and for a long time enjoyed a considerable vogue; but the high mortality due to the toxicity of cocaine caused it to be regarded as dangerous. The substitution of the less toxic tropacocaine, which was the best drug for this purpose until the introduction of procaine, greatly lowered the mortality. Indeed, Ollendorff (1917) was able to report 14,741 cases with a mortality of 0.076 per cent from tropacocaine.

Physiological conditions in spinal anesthesia. The widespread usefulness of spinal anesthesia is rendered possible by the fact that, when the local anesthetic is administered by this route, failure of the respiratory center is rarely brought about. In spinal anesthesia, circulation fails before the respiration. This is in striking contrast to the toxic reactions following intravenous injection, or from absorption after infiltration or nerve block anesthesia, for in those cases the respiratory center fails before the circulation.

Difference of manifestation. This is due to the fact, emphasized by Wayne Babcock (1928), Koster (1928) and particularly by Gaston Labat (1928), that, as the local anesthetic spreads along the spinal canal it blocks the thoracic roots. V. Brunn (1922) believes that the pressure of the cerebrospinal fluid tends to force it and the local anesthetic, which it contains, outward along the nerve roots. The drug thus paralyzes the sympathetic vasoconstrictor nerves, all of whose rami communicantes arise from this part of the spinal cord, before the drug reaches

the level of the respiratory center in the medulla. This paralysis of the vasomotors causes a tremendous fall of blood pressure accompanied by shock and anemia of the brain, which in itself sometimes results secondarily in failure of the respiration.

Labat (1928), Pitkin (1928) and others have shown that fifteen to thirty minutes after spinal anesthesia the procaine can be found only in minute traces in the spinal fluid taken from the level of the thoracic, while very positive tests are given by the fluid from the lumbar region. After 35 minutes very little remains free in the spinal fluid. Klose and Vogt (1909) had made similar observations with tropacocaine. This indicates that the procaine is adsorbed by the nerve tissue so rapidly that very little of it is left to reach the medulla.

However, the medulla and respiratory center are not as sensitive as has been generally assumed. Koster and Kasman (1929) state that in a series of 750 cases of spinal anesthesia they did not once encounter a single case of serious respiratory difficulty. They claim that application of 0.25 per cent procaine directly to the medulla of frogs and 2.5 per cent procaine to the medulla of a cat caused no change in respiration. Their observations have been confirmed by Harrison (1931) who found that procaine solutions introduced between base of the skull and the 1st cervical spine of cats did not cause respiratory paralysis. However, when the solution was made to gravitate anteriorly so as to come into close contact with the roots of the phrenic nerve (by increasing its specific gravity with 5 per cent ammonium citrate and 5 per cent sodium ferrocyanide) respiratory paralysis resulted. On the other hand, this could be prevented by turning the animals on their backs so that the weighted solutions would gravitate to the posterior roots. Koster and Weintrob (1928) found that guinea pigs injected intraspinally with procaine solution could be placed in the Trendelenburg position for one-half hour without causing respiratory failure; and Harrison hung two goats up by their heels after spinal anesthesia for half an hour with similar lack of deleterious effect.

Control of blood pressure in spinal anesthesia. The fall of blood pressure can be controlled by the administration of the directly vasoconstricting drugs such as epinephrine and ephedrine; and particularly by keeping the patients in the Trendelenburg position with head and shoulders lowered and pelvis raised. In the earlier years of spinal anesthesia this position was scrupulously avoided for fear that the drug would gravitate into the medulla and paralyze the respiratory center. Dönitz (1906), Bier (1909) and Gauss (1909) showed, however, that it could

be used with impunity and even with benefit; and the more recent studies of Babcock, and numerous others have confirmed their observations.

Fall of blood pressure can usually be prevented by the routine preliminary administration of 25 to 50 mgm., or in low blood pressure cases even 100 mgm. of ephedrine sulphate subcutaneously or intramuscularly. Ockerblad and Dillon (1927), Rudolf and Graham (1927), Babcock (1928), Labat (1928), Pitkin (1928), Evans (1928), Wenzel (1930), Kelly (1930), Chute (1931), Bessessen (1930) usually give about 60 mgm. ephedrine hydrochloride or sulphate; but occasionally administer as much as 120 mgm. or even 150 mgm. For collapse Evans gives 1 mgm. epinephrine intramuscularly and Kelton (1930) uses intravenous injections of both ephedrine and epinephrine.

Wangensteen, who has obtained excellent results, remarkably free from complications, in the surgical clinic of the university of Minnesota, has been placing the patient routinely in the Trendelenburg position before beginning the operation. He also administers 50 to 100 mgm. of ephedrine salts fifteen minutes before the spinal puncture. He frequently elevates to a Trendelenburg inclination of 45 degrees. After operation the foot of the patient's bed is kept elevated one foot for several hours. Among 772 operations under spinal anesthesia one death attributable to the anesthetic occurred. This patient was in a desperate condition from an old perforated duodenal ulcer.

Pathological changes induced by spinal anesthetics. When toxic amounts of local anesthetics are injected, definite histologic changes in the nerve cells can be detected. Spielmeyer (1908–1931) found chromatolysis, dissolution of Nissl bodies and of the nuclear membrane in the ganglion cells after intraspinal stovaine in dogs. This was accompanied by lesions of the columns of Goll and Burdach, because the drug seemed to enter the nerve fiber at the point where the dorsal roots enter the cord, i.e., in the region where the myelinization is especially thin. Similar observations have been made by Van Lier (1922) and by Loyal Davis (1931). However, Babcock (1922), states that in 15,000 cases of spinal anesthesia he has seen "no case of secondary degeneration" of the cord, even after repeated anesthesias (up to 12 times) on the same patient, though "secondary functional symptoms are not uncommon."

Technique for spinal anesthesia. For producing spinal anesthesia the needle may be introduced into any of the intervertebral spaces from the fourth to the first lumbar, the level at which it is introduced and the

amount of anesthetic introduced determining to a large degree the level to which the zone of anesthesia will reach.

While inserting the needle Evans keeps the patient on his side with his knees drawn up against his abdomen. The needle should have a short and sharp bevel (18 to 20 gauge) introduced with a stylet, and should be inserted in the midline at right angles to the skin. In order to direct the needle at exactly the proper angle Harrison (1931) uses a special metal director. This consists of a thick strip of metal through which a narrow tube is soldered at just the proper angle to enable the needle to be introduced with certainty and speed.

Pitkin (1928) has shown by numerous experiments on cadavers that the average capacity of the spinal canal is as follows: To the level of the iliac crests 4 cc.; to the level of the costal margin 6 to 7 cc.; to the xiphisternal level 8 cc. When cerebrospinal fluid is removed and these amounts injected with the patient in the horizontal position, the drug may therefore be expected to reach without diffusion to the level corresponding to the amount used.

Lundy (1931) claims that the intensity and duration of the anesthesia is proportional to the percentage concentration of the drug dissolved in the cerebrospinal fluid injected, rather than to the total amounts used. Thus, 150 mgm. procaine dissolved in 2 cc. cerebrospinal fluid gives more lasting anesthesia than when dissolved in 5 cc.; and 200 mgm. dissolved in 2 cc. may cause a post-operative dragging of the legs in man, whereas this result is not obtained when the same amount of drug is dissolved in a larger amount of fluid.

For anesthesia of special areas, Evans removes and reinjects the following amounts into the following levels.

1. Perineum, leg, and foot, 5th lumbar interspace, withdraw 1 to 2 cc. spinal fluid to dissolve the drug (procaine), draw out 1 cc., inject 1 cc., aspirate, then inject the whole amount very slowly and uniformly.
2. Thigh and knee, fourth lumbar interspace, but for the last dilution withdraw and inject 3 cc. of the spinal fluid.
3. Groin and buttocks, fourth interspace, using 4 cc. instead of 3 cc.
4. Lower abdomen and umbilical region, third lumbar interspace, 6 cc. spinal fluid.
5. Upper abdomen, second lumbar interspace 8 cc. spinal fluid.
6. Koster (1928) and Koster and Kasman (1929) claim to have used spinal anesthesia with impunity for more than 200 operations on the head and neck, such as thyroidectomies and even mastoidectomy. In spite of their favorable reports, most surgeons believe that spinal

anesthesia should not be used for operation above the level of the abdomen.

The amount of drug necessary to induce anesthesia is nearly proportional to the amount of cerebrospinal fluid used, ranging with procaine from 50 to 300 mgm., with a maximum of 400 mgm. (Pitkin).

The amount of drug injected also determines the duration of the anesthesia. Thus Pitkin records the following effects: 1.324 cc. spinocaine (0.130 gram procaine) 30 to 45 minutes; 1.65 cc., (0.16 gm.) procaine 45 to 75 minutes; 2.0 cc. (0.195 gram procaine) 2 hours.

When the procaine or other drug is dissolved in the spinal fluid the specific gravity of the latter is slightly increased above its normal (1.0065, Babcock, 1928) and it, therefore, tends to gravitate downward. The level to which the anesthetic will reach can be regulated by altering the patient's position.

Alterations of the specific gravity of solutions injected. In order to localize the spinal anesthetic and to prevent its spreading upward along the cord, efforts have been made to increase or decrease greatly the specific gravity of the anesthetic solution injected. When heavy solutions are used, the patient's shoulders should be propped up with pillows, eight inches high for perineal anesthesia, five inches high for upper abdominal anesthesia (Harrison, 1931). When solutions lighter than cerebrospinal fluid are used, the Trendelenburg position is preferable; though as stated above this is also the optimum position even if the drug is dissolved in cerebrospinal fluid alone, and thus has a specific gravity very slightly above that of the cerebrospinal fluid itself. Even when the heavier solutions are used, the danger of low blood pressure and collapse from paralysis of the vasomotor fibers remains when the patient's shoulders are elevated.

Erhardt (1908) increased the specific gravity of the tropacocaine solutions which he used, by the addition of gum arabic and egg albumin. Glucose, mannitol, gelatine and other substances have been tried for the same purpose (Babcock, 1928). Harrison (1931) prefers 10 per cent cane sugar. Romberger (1930) uses 40 per cent dextrose.

Since 1908 anesthetic solutions have been made lighter than cerebrospinal fluid by the addition of 10 per cent of alcohol and the patient is then kept in the Trendelenburg position.

Pitkin (1928) with the collaboration of C. W. Hooper, after a large series of experiments on models, cadavers and dogs, was able to improve this method greatly and prepared two mixtures "spinocaine," heavy and light, in which the procaine would gravitate either downward or

upward and would not mix with the rest of the spinal fluid for over two hours, i.e., until after the drug had been adsorbed upon the nerves and spinal cord.

Spinocaine no. 1 which is of lower specific gravity (1.005) than the cerebrospinal fluid (1.007) is the one most usually employed. Its composition is as follows:

Strychnine sulphate	0.0022	gram
Novocaine	0.195	gram
Starch paste (mixture of starch and gliadin, "amylo prolamin")	0.13	gram
Alcohol	0.324	gram
Normal saline	2.0	cc.

The strychnine is introduced to stimulate the vasomotor nerves. In the heavier solution "spinocaine heavy" the alcohol is omitted.

Pitkin has shown that using the light spinocaine the procaine can be localized very sharply. With this preparation the patient is kept in exactly the opposite position from the usual one, i.e., in the Trendelenburg position at an inclination of 15° with hips elevated, and head down. The level to which the drug ascends is regulated by the inclination of the body as well as by the amount of drug and of spinal fluid used.

This method with the details of technique worked out by Pitkin is somewhat safer than the simple solution of procaine in cerebrospinal fluid. As much as 0.400 gram procaine has been used with safety.

Schwyzer (1930) finds that by turning the patient on his stomach immediately and keeping his head down the spinocaine will rise only into the dorsal curvature of the spine, and the stomach, gall bladder and upper abdomen can be anesthetized with 2 to 3 cc. spinocaine (0.195 to 0.283 gram procaine).

The literature of Pitkin's technique is reviewed by Kelly (1930) and by Gallagher (1931).

Pitkin's procedure is unquestionably simple, sound in principal, and may be used successfully by surgeons having less experience than is necessary for simply dissolving procaine in the spinal fluid. C. W. Hooper informs the writer that in over 200,000 cases the results have been most satisfactory and very few deaths have been reported. Two deaths have been reported by Demel (1931) where high spinal anesthesia has been used.

It must be admitted, however, that recently the use of simple solution of procaine in spinal fluid has been becoming more general than that of

spinocaine, or of solutions whose specific gravity differs greatly from that of the spinal fluid. Spinocaine itself usually requires a larger, and, therefore, more toxic amounts of procaine, than are necessary when this drug is used alone.

Other procedures for diminishing toxicity in spinal anesthesia. Siebert (1912) and Laewen (1913) used procaine alkalinized with sodium bicarbonate according to the method of Gros (see p. 198) to increase the action of the procaine, and report satisfactory results though others do not find that this gives better results than procaine itself.

Matous (1924) adds 0.24 to 0.42 gram caffeine sodium benzoate to the procaine before dissolving it in the spinal fluid. He believes that this decreases, but does not abolish bulbar symptoms. It is possible also that it may give a synergistic action with procaine, as shown in the experiments of Lipschitz, Laubender and Weingarten (see p. 208).

Bloch and Hertz (1921–1923) injected 0.1 gram caffeine intraspinally to stop collapse after procaine and Jonnesco (1923) claimed to have had good results from the addition of 0.25 gram caffeine to the stovaine which he used in 523 cases. On the other hand Guibal (1923) found no advantage in the addition of caffeine and even regarded it as positively dangerous.

Spinal anesthetics other than procaine. The introduction of newer local anesthetics within the past few years has led to their use in spinal anesthesia. Among those giving evidence of usefulness are tutocaine, Braun (1924), who suggests 0.6 to 1. cc. of 0.25 per cent solution, equivalent to 1.5 to 2.5 mgm.; nupercaine, Keyes and McLellan (1931), who use 2 cc. of a 1:200 solution, equivalent to 10 mgm.; and pantocaine, Lundy and Essex (1931), who use 1 mgm. per 10 pounds of body weight plus an extra 5 mgm. with a maximum dose of 25 mgm. Both nupercaine and pantocaine produce anesthesias of much longer duration than does procaine.

Saligenin, in spite of its low toxicity, is of no value in spinal anesthesia because of the short duration of its effects. In dogs spinal anesthesia from saligenin lasts only 10 to 12 minutes (Hirschfelder and Dvorak, 1931).

The therapeutic coefficient, i.e., the ratio between

$$\frac{\text{Minimal lethal dose}}{\text{Minimal amount of anesthetic necessary to produce anesthesia}}$$

for the various local anesthetics introduced intraspinally has not yet been determined in any adequate series of mammals, but Bieter, Harvey and

Burgess (1931) have determined this for summer frogs. Their results are given in the table below:

Minimal anesthetic and minimal lethal doses spinal anesthesia in summer frogs

DRUG	MINIMAL ANESTHETIC DOSE	M.L.D.	RATIO $\frac{\text{M.L.D.}}{\text{M.A.D.}}$
	mgm. per gram	*mgm. per gram*	
Nupercaine	0.0036	0.004	1.1
Pantocaine	0.0045	0.02	4.4
Butyn	0.008	0.03	3.7
Alypine	0.01	0.04	4.0
Neothesine	0.01	0.05	5.0
Tutocaine	0.015	0.2	13.3
Stovaine	0.02	0.045	2.25
Cocaine	0.035	0.175	5.0
Procaine	0.045	0.22	4.88
Saligenin	0.125	0.5 (not toxic)	

Since metabolism and rate of destruction and ratio of elimination of drugs in mammals differ from those of frogs it is evident that these data cannot be regarded as an exact index of clinical conditions, but these therapeutic indices probably are of considerable importance in evaluating various spinal anesthetics.

Mortality in spinal anesthesia. The results in the literature are very conflicting as regards operative risk and mortality in the hands of different operators.

	CASES	DEATHS	DEATH RATE
Tomachewski			1:17,887
Strauss			1:2,524
Hohmeier and Konig			1:200
Mayer	3,310	5	1:550
Franz	3,355	6	1:479
Babcock (before 1914)	6,000	12	1:500*
Babcock (before 1928)	6,000	No deaths	
Koster and Weintrob (own cases)	6,000	9	1:667
Koster and Weintrob from literature	44,241	29	1:1,525
Koster (1928)	2,500	No deaths attributable to anesthetic	
Case (1928)	1,100	No deaths	

* Mostly hopeless cases.

Other large series of spinal anesthesias have been reported by Boyd and Yount (1917), Desplas and Mellet (1918), Wells (1920–1927), Morrison (1921), Babcock (1922–1928), Kroenig (1925), Broglio (1926), Campbell (1927), Ducuing (1927), Rapaport (1928), Brunn (1929), Jackson (1930), Kelton (1930).

As many of the cases chosen for spinal anesthesia have been in a condition too poor for general anesthetics these mortalities acutally do not give a true picture of the natural operative risk. For example, the 25 deaths which were attributable to causes other than the anesthetic were excluded from the 44,241 cases quoted from the literature by Koster and Weintrob, the 4 remaining deaths definitely due to the anesthetic would make the mortality only 1 in 11,000. The latter figure is probably too low, however, and Koster and Weintrob's own results, one death in 667, probably represent a fair average mortality for cases, many of which are in a dangerous condition before the operation. It is difficult to obtain figures which represent a true picture of the actual danger from spinal anesthesia; as on the one hand, many deaths which could be ascribed to the anesthetic are not reported, and on the other hand, many conscientious surgeons who report all their deaths include cases which would have died no matter what anesthetic procedure had been used.

Evans believes that "the mortality is less than with general anesthesia varying in different statistics from 1 to 500 to 1 in 10,000 cases." Babcock believes that the former rate may be expected on unselected cases, the latter if the spinal anesthesia is performed on only carefully selected ones.

Headache from spinal anesthesia. Besides actual death or collapse spinal anesthesia is often followed by headache, which may last from two days to a week. According to Evans this is due to alteration of intraspinal pressure, irritation of the dural fibers, etc. Bagley (1928) believes that they are usually caused by intraspinal hemorrhage.

Koster (1928) is able to relieve most headaches by dehydrating the patients with enemas of 180 cc. 50 per cent $MgSO_4$ solutions, or by spinal puncture and drainage. Harrison (1931) accomplishes this by intravenous injections of hypertonic (5 per cent) NaCl solutions. He finds that this not only relieves the headache but also accelerates the termination of the anesthesia, stopping tutocaine anesthesia within five minutes, procaine anesthesia within twenty minutes. He regards this as evidence that the procaine is more firmly adsorbed on the nerve fibers than the tutocaine.

Table of Anesthetic and Mi

(Bold-faced numerals indic

Drug	Threshold Anesthetic Concentrations in per cent					Duration of Anesthesia in minutes																
	Frog				Rabbit's Cornea				Human Intracutaneous							Frog	Mouse					
	Skin	Sciatic 3-30 min. method BK-12 hr. – Motor	Sciatic 3-30 min. method BK-12 hr. – Sensory	Rabbit's Cornea	Human Intracutaneous	1%	Other %'s	Anesthetic Cocaine Index	0.125% Without epinephrine	0.125% With epinephrine 1:100,000 S 1:150,000 Me	0.5% Without epinephrine	0.5% With epinephrine 1:50,000	Anesthetic Cocaine Index			Subcutaneous	Intravenous					
Cocaine	0.1 U 0.125 S – N	0.05 BK 0.125 S – M	0.01 BK 0.1 0.125 0.125-0.25 F 0.25 M – RC	0.02 U 0.25 Co – F – W 0.5 S – M – Ko **0.32**	0.005-0.01 Rb 0.0125 Ko – W 0.05 N 0.031 S – Me 0.033 H **0.0206**	18 Co – LL 20 S 24 RC 26 Ro 27 Ry **22**	.2% 26 SL 35 RC	1 Co – So – SL	6-15 So 10 Me	120 So 195 Me	10 NL – Rh 10-18 So **12**		1 So	0.42 Fi 0.45 Ro 0.78 LB 1.00 R **0.66**	0.10 D – F – R 0.15 H – Me – SL 0.15-0.18 RC 0.20-0.35 Gu 0.25 Me 0.30 Co 0.375 LB **0.189**	0.03- F					
Psicaine		0.25 BK	0.01 BK 0.1 Go	0.25 W	0.016 Ro – W		0.5% 7.5 RO		9 W								0.33 Go					
Tropacocaine	0.05 N 0.062 S	0.035 BK 0.125 S – M	0.01 0.125 0.25 BK N	0.5 Co 2.0 S – M **1.5**	0.0125 Ro 0.03 N 0.031 S **0.0245**	0 S 14 Co		0.23 So 0.80 Co	5-7 So	10 So	5-10 So		1 So	1.3 LB		0.05 LB						
β Eucaine		0.05 BK	0.01 BK	1.0 Co – S	0.0206 Ro 0.062 S **0.0414**	10 Co 30 So		0.5 Co – So	5-8.5 So	70 So	15-17 So		0.5 So	1.3 LB		0.78 LB						
Procaine (Novocaine)	0.015 F 0.25 M 0.5 S 1.0 U	0.075 BK 0.125 S	0.05 BK 0.25 S – F 0.5 M	0.1 U 1.0 Ko 2.0 F 5.0 Co 8.0 S – M **4.0**	0.01-0.025 Rb 0.025 Ko 0.03-0.06 N 0.031 S 0.0333 Ro 0.061 Me 0.07 H **0.038**	0 Co – S – Sc 0? Ry 0.5 Mo	2% 10 F 0 SL	0.05 Co 0.00 So	0.5-10.5 So 10 Me	105 So 130 Me	9 NL 9-14 So 10 Rh 13.5 Wi 16 Er 20 Me 25 Bo **16**	171 Be	1 So	0.7 R 1.2 Ro 2.0 LB **1.5**	0.55 R 0.60-0.70 F 0.65 LB 0.8 RC 0.90 Me – SL 1.00 Mc 1.10 Co **0.80**	0.06-0.07 F					
Isocaine					0.031 Me		2% 25 SL	0.9 SL	12 Me	190 Me						0.40 SL						
Butyn	0.062 BS	0.015 M 0.062 BS	0.125 BS	0.125 Co 0.5 BS **0.312**	0.015 BS – M 0.031 Me **0.020**	30 Co	2% 40 SL	1.4 SL 2.0 Co	15 Me	100 Me	45 Be	200.5 Be				0.10 SL 0.15 Co – D						
Tutocaine	1.0 U	0.025 BK	0.0075 BK	0.031 W 0.06 U 1.0 Co **0.363**	0.0031 Ro – W	6 Co 18 Ry 37 Jc **20**		0.33 Co	11 Sh 15 W		18.5 Wi 20.0 –					0.35 F 0.5 Co	0.05 F					
Larocaine		0.065 Di	0.08 Di	0.12 F 0.125 Di	0.03 Di	60 F	0.12% 14 F									0.30 F	0.04 F					
Panthesine		0.05 BK	0.01 BK	0.01 Ro 0.066 Ro **0.038**	0.005 Ro	26 Ro	0.1% 9 RO								0.84 Ro	0.3-0.35 Ro						
Pantocaine							0.01% 12 ? FS 0.1% 60 SB				25 Wi 20 Er					0.04 FS	0.008 FS					
Neothesine			0.25 RC		0.0077 Ro	22 RC	2% 34 RC		16 Me		25 Me 27.5 Be	140 Be				0.8 RC 0.9 Mc		1.3				
Apothesine				2.0 Co	0.033 H 0.25 S **0.141**		2% 4 Co	0.125 So					0.125 So			0.7 H						
Alypine	0.05 M 0.062 S	0.05 BK 0.125 S 0.167 M	0.0075 BK 0.25 S 0.5 M	0.5 Co 2.0 S – M **1.5**	0.03 M 0.031 S 0.0375 Ro **0.049**	? Co 10 So		0.25 So 0.7 Co	0.25-12 So	90 So	10 So		1 So	0.25 I 0.39 LB		0.26 LB		0.2+ 0.43				
Stovaine		0.01 BK	0.0075 BK	2.0 Co	0.0161 Ro	? Co	2% 12 Co	0.25 Co	5-7 So					0.975 LB		0.17-0.19 L 0.52 LB						
Acoine					0.0073 Ro																	
Nupercaine	Below 0.1 U	0.004 Ro	0.005 Ro	0.001 U 0.005 Ro **0.003**	0.0025 Ro		0.01% 51.5 LL 0.1% 130 SB				35 Wi 100 Be	221.5 Be		0.06 LL								
Phenacaine (Holocaine)				0.125 Co 0.25 BS 0.5 N **0.291**	0.0063 Ro	30 S 31 Co		1.0 So 2.5 Co														

Minimal Lethal Doses

indicate averages)

Toxicity
(M.L.D. in gm. per kg.)

Rat		Guinea Pig		Rabbit		Cat		Dog	Toxic Co-caine Index	Therapeutic Coefficients of Hoffman Kochmann (HK) *Determined from average figures from this table*	
Subcutaneous	Intravenous	Subcutaneous	Intravenous	Subcutaneous	Intravenous	Subcutaneous	Intravenous	Subcutaneous		Surface Anesthesia Rabbit's cornea	Intracutaneous Anesthesia Human
0.10 I 0.15-0.18 A 0.20-0.30 JC 0.25 R " RK " SL 0.37 NH 0.40-0.60 BJ **0.277**	0.0125 HB 0.0175 RC 0.03 Go (slow injec.) (0.05 M:B 0.07 RC Intraperit.)	0.02 - 0.04 A 0.038 - 0.062 Bu 0.045 Fi 0.05 Cl " H " HK " K " Ko " W 0.05 - 0.06 Gr " Vi 0.06 Co " R 0.08 I **0.05**	0.02 K (0.06 K intraperit.)	0.05 FJ 0.09 - 0.10 Ri 0.07 F 0.075 R 0.09 SL 0.10 An " Gr " HL " CB " F 0.10 - 0.12 Vi 0.10 - 0.15 I 0.12 Au 0.15 - 0.3 A 0.16 Fi 0.18 LB 0.20 Ch " NH " Ri **0.126**	0.0034 Gr 0.0077 R 0.01 MB 0.01 - 0.022 Bu 0.012 NH 0.017 RC 0.018 FJ 0.02 - 0.025 F **0.0149**	0.02 An 0.02 - 0.04 A 0.03 Fi " Go " Gr " I " SL 0.01 - 0.04 BH 0.05 NH **0.0319**	0.01 H 0.015 Ch " Co " MB 0.018 BH **0.0146**	0.01 - 0.025 A 0.02 - 0.04 I 0.022 - 0.03 Gi 0.0261 T 0.03 Ho " Mo 0.03 - 0.07 B 0.033 N 0.035 Fi 0.045 NH 0.05 - 0.07 Bi **0.035**	100 Ko " SL	**0.15** (1 ..... SL, RC Therap. Index of SL)	**2.4** (1 ..... RC Therap. Index of SL)
	0.03 Go (slow injec.)	0.09 Q " K 0.10 Ko " W **0.095**	0.02 K (0.2 K intraperit.)			0.06 Go	0.015 Go		50 Ko	**0.38**	**5.9**
	0.015 - 0.020 MB	0.20 Cl " HK " Ko 0.27 K **0.217**	0.025 K (0.17 K intraperit.)	0.32 LB 0.5 Ch			0.018-0.022 EH		25 Ko	**0.14**	**8.8**
	0.015 - 0.025 MB	0.25 K 0.30 HK " Ko 0.30 - 0.35 Vi 0.40 Cl **0.31**	0.03 K (0.18 K intraperit.)	0.4 - 0.5 Vi			0.010-0.0125 EH		16 Ko	**0.31**	**7.4**
1.0 - 1.25 BJ 1.6 SL 1.8 - 2.0 Sc 2.0 R 2.1 RC **1.65**	0.045 - 0.055 MB 0.050 Mc 0.053 RC (0.30 RC Intraperit.)	0.35 + Co 0.40 Cl " HK " Ko " W 0.4 - 0.42 Ko 0.55 K 0.6 R **0.43**	0.05 K " Ko (0.6 K intraperit.)	0.35 - 0.30 Mu 0.35 - 0.40 Bi 0.40 FJ " R 0.42 G 0.45 P " Ho " Ko 0.60 Co " SL 0.73 Hu **0.46**	0.03 R 0.04 Ko 0.04 - 0.055 F 0.04 - 0.070 Sc 0.055 FJ 0.055 - 0.065 MF 0.06 Ho 0.065 Mu 0.09 Cb **0.055**	0.45 SL	0.04 - 0.045 EH	0.25 Bi	12.5 Ko 13.0 SL	**0.107**	**11.3** (3.8 ..... RC Therap. Index)
0.70 SL	(0.10 M:B Intraperit.)			0.30 SL		0.20 SL			29 SL	(3.0 ..... SL Therap. Index)	
0.15 SL 0.19 NH 0.20-0.25 BJ **0.197**	0.0075 - 0.01 MB	0.07 + Co		0.050 SL 0.055 NH		0.010 SL 0.055 NH	0.015 Ha	0.055 NH	149 SL	**0.22** (1.0 ..... SL Therap. Index)	**3.5**
		0.116 - 0.174 BW 0.12 - 0.15 Co 0.17 - 0.34 Hi 0.20 Ko " W 0.27 K **0.193**	0.03 K (0.25 K intraperit.)	0.2 F 0.3 Co	0.015 F 0.021 Co			(0.015 - 0.02 Sh intraven.)	25 Ko	**0.53**	**62.2**
		0.2 Di		0.15 F	0.015 F					**1.6**	**6.6**
		0.075 Ko 0.15 Ro **0.112**	0.02 Ro	0.24 Ro	0.02 Ro					**2.9**	**22.4**
				0.02 FJ	0.006 FJ						
1.3 RC	0.0175 Mc 0.02 RC				0.028 RC					(2.8 ..... RC Therap. Index)	(4.27 ..... RC Therap. Index)
		0.25 H			0.37 - 0.48 MF		0.02 EH 0.025 H			**0.125**	**1.77**
0.2 + I 0.43 Sc	0.01 - 0.015 MB	0.06 Cl " I 0.07 HK " K 0.075 Ko 0.1 Sc **0.072**	0.015 K 0.02 Sc (0.1 K intraperit.)	0.05 I 0.11 Sc 0.13 LB **0.096**	0.01 Sc	0.06 I " Sc	0.01 EH " Sc	0.07 I	66 Ko	**0.048**	**1.47**
	0.025 - 0.03 MB	0.16 K 0.18 - 0.2 L 0.2 Cl 0.25 HK " Ko **0.21**	0.01 Sc 0.04 K (0.23 K intraperit.)	0.15 - 0.17 Bi " L 0.18 Ba 0.25 LB **0.178**	0.009 Sc 0.025 - 0.05 Ba 0.03 SH **0.0285**		0.008 Sc 0.025 - 0.03 EH 0.03 SH **0.023**	0.1 - 0.12 L 0.15 Bi	20 Ko	**0.105**	**12.5**
		0.15 HK " Ko							33 Ko		**21.9**
		0.0075 U 0.015 Ko **0.0112**		0.005 LL 0.01 - 0.035 U	0.0024 LL 0.0025 - 0.0045 U					**3.73**	**4.48**
		0.05 HK " Ko 0.06 K **0.053**	0.015 K (0.05 K intraperit.)				0.01 EH		100 Ko	**0.18**	**8.4**

Nausea and vomiting may also occur. With the improvements in technique cited above, these unpleasant features are much less frequent than after general anesthetics.

Prophylactic premedication. The toxicity of the drug both as regards mortality and unpleasant symptoms can be greatly reduced by the routine preliminary administration of barbitals (see p. 246, also Evans, 1928, Sise and Woodbridge, 1930). Grant (1930) and Thorek (1930) give morphine and scopolamine before the operation.

Spinal anesthesia in non-surgical conditions. Spinal anesthesia can produce symptomatic relief in non-surgical conditions. Markowitz and Campbell (1927) have brought about relief of experimental ileus with spinal anesthesia, and Chenut (1927), Neely (1930) and Spencer (1930) claim similar results in clinical cases.

Marinisco, Kreindler and Facon (1930) also claim that the intraspinal injection of 70 mgm. procaine can inhibit Parkinsonian rigidity for half an hour.

Caudal block. The branches of the entire sacral plexus and cauda equina can be anesthetized without penetrating the dura mater by caudal block anesthesia. The sacral hiatus is a large space easily located by its prominent edges in the midline between the sacral and coccygeal cornua. Through this the needle is passed, with the patient lying on his stomach and a pillow under his hips. According to Labat who gives a good description of the technic 30 to 40 cc. of 2 per cent procaine should be injected slowly.

Like transsacral and parasacral anesthesia caudal block is relatively free from the unpleasant features of spinal anesthesia; and a greater number of nerves is anesthetized than when presacral block is used, though it is about equivalent to transsacral block.

Intravenous and intra-arterial anesthesia. Bier (1908) anesthetized the limbs by injecting local anesthetics directly into the veins (brachial, median, cephalic, saphenous) after rendering the limb bloodless and thus preventing venous return with Esmarch tourniquets but this procedure has no advantage over the simpler methods.

Still more can this be said of the intraarterial anesthesia in which the drug (½ to 1 per cent procaine) is injected directly into the artery after applying an Esmarch tourniquet (v. Oppel, 1909; Goyanes, 1909).

TABLE OF ANESTHETIC AND MINIMAL LETHAL DOSES (see pages 262 and 263).

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CONSTITUTION AND DISEASE

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The present continental revival of interest in the problems of constitution as related to medicine—and particularly resistance to disease—is interesting for a variety of reasons.

Submerged ever since the middle of the last century by the overwhelming accumulation of exact knowledge derived from the intensive study of anatomy, physiology, pathology, bacteriology, hygiene and immunology, the interest in constitution has followed a bypath quite out of the range of the midday sun that has illuminated medical progress. But the internist and the pathologist have returned to an interest in constitution because they now deal less frequently with the acute infectious diseases and more often with ailments wherein the influences of the condition of the patient play a more obvious rôle. These include cancer—"the wear and tear diseases"—insanities—and even tuberculosis is by no means a closed chapter. In all of these the constitutional factor seems of greatest importance. Rössle's work, too, has shown that constitution is the chief factor as far as death in old age is concerned and that longevity is distinctly an inheritable factor.

The modern study of genetics, contributing so much to our accurate knowledge of constitutional factors, has played a distinct rôle in the re-awakening of medical interest. So, too, has the growing knowledge of endocrinology. Anthropology has been a stimulus. The scientific study of dietetic and social and climatic factors and their influence on the individual has also been of help. We begin to have a firm foundation for what originally may have seemed clinically obvious, but which later seemed scientifically unsound.

Whether or not the modern study of constitution may be useful in the process of assorting and ordering our medical knowledge no one can foretell.

HISTORY. The pathology of the ancients was a constitutional pathology: "The body should not be too thin; favorable a square chest with generous growth of hair, the framework well fleshed. One who possesses such characteristics has the greatest chance of recovery. On the other

hand he who possesses none of these characteristics will die the sooner." In so simple and straightforward a fashion do we find Hippocratic prognosis recognizing the constitutional influence in tuberculosis.

This constitutional pathology (Galen) dominated clinical medicine through the ages until Morgagni's epochal text began to fix disease in definite organs. The final coup de grace came with young Virchow's devastating review of Rokitansky's *System of Pathology* in 1846.

But the clinical concept of constitution as a factor of resistance persisted both in France and in England while in the German School a reawakening of interest came only with the turn of the century. The German school has since contributed many thorough studies and to Rosenbach, Hüppe, Gottstein, and particularly Martius, we owe the development of the modern scientific clinical studies in constitution. The pathologists Benecke, von Hansemann and Bartel contributed, and from the school of Tandler have come most valuable studies. Italy contributed the work of de Giovanni and his pupils Pende and Viola. They have developed in great detail the field of "habitus." France gave us the concept of arthritismus (Bazin, 1865), the classification of Sigaud as well as the excellent statistical studies of the military surgeons.

To the more recent contributions reference will repeatedly be made in this review.

DEFINITIONS. Martius defined constitution as the somatic aggregate (inclusive of irritability and liability to disease) that remains the relatively unaltered possession of the individual, with the characteristic reactivity of the organism more or less permanently fixed.

While this concept is not accepted by all, its broad outline coincides with the general medical aspect of the constitutional problem—the phaenotype, i.e., the "condition" in contradistinction to the strictly limited genotype.

In medicine we deal less with abstractions, rather with concrete problems. The term constitution must embrace therefore not only the quality of the protoplasm in its form and structure, but the functional status as made apparent in physiological and pathological reactivity—in adaptability to change in environment—in modifications produced by season, climate, diet, age, endogenous and exogenous irritants, disease, physical and mental trauma, and the ever varying forces of the universe of which the individual is a part.

As a consequence, constitution, from the medical point of view, can never be regarded as static. But it is herein that we find the germ of scientific skepticism. Even biological science deals preferably in well

defined units. But by constitution we indicate a labile and therefore intangible quantity which in its very nature the scientist must question, as Germain Sée did in his characterization that "La disposition est un mot qui masque notre ignorance." But if we try to express constitution strictly in terms of morphology, in terms of fixed dimensions, indices and ratios, we promptly rule out medical interest and usefulness.

Borchardt defines constitution as the status of the organism as conditioned by heredity and environment. It is the totality of the individual, in structure, form, appearance, physical and psychological ability, reactivity, the condition of nutrition, the resistance to infections and intoxications, the psychic makeup, irrespective of the contingency of the individual phenomena on heredity or environmental influences. And Draper defines human constitution as "that aggregate of hereditarial characters, influenced more or less by environment, which determines the individual's reaction, successful or unsuccessful, to the stress of environment. But it is not always easy to decide which of the so-called inherited values are true germ plasm transmissions." It is obvious that the reactivity of the organism begins to dominate the recent conceptions.

Before proceeding to a discussion of various classifications it may be well to define briefly the terms more commonly used in the literature.

It is agreed that two fundamental groups make up the components represented in the individual, i.e., the inherited germ plasma and the forces that modify that germ plasma after fertilization.

The genotype (Johannsen) or the idiotype (Siemens) denotes the former and is to be placed opposite the merely external appearance and the acquired peculiarities of the so-called paratype of Siemens. Under the term bodily condition one understands a combination of the genotype with all its acquired modifications and phaenotype is used to denote this sum total in the final product.

We speak of a constitutional disturbance if some individual characteristic falls far out from the range of the normal, as for instance, a peculiar disposition to a disease. But when we speak of disposition we must keep in mind that the tendency to a particular disease may be diminished as well as increased. When a tendency to a series of clinical phenomena is apparent, rather than to a specific disease, we speak of a diathesis.

THE NORMAL. While clinical constitutional science is interested largely in the deviations from the normal we must first of all make some effort to define normalcy. Kaup as well as Rautmann have sought to delineate and to correlate many of the characteristic properties of the

body and its parts—the build, form, etc., and there has been collected a large statistical material in the anthropological, military, as well as constitutional literature dealing with both juvenile and adult groups. The material involves, of course, largely the measurement of form rather than function and mathematical treatment of it has followed the methods laid down by Galton, Pearson and Fechner.

Because of the importance of the length of the body a fundamental correlation for a variety of comparisons is made on the basis of "x times 100 divided by the length." For instance, the Rohrer index is weight times 100 divided by the length, cubed. The Kaup index is weight divided by the length squared. One of the most useful indices, that of Pignet, is length minus (body weight plus chest circumference). Another index similar in character is that of Brugsch—chest circumference times 100, divided by the body length.

Of far greater importance is the use of statistical material to determine the range of individual variability. Quetelet was the first to develop the field.

It is, of course, impossible to define a normal type and we cannot accept the average of many measurements as an index of normalcy, we may merely fix the range of deviation and determine the factors of variations of different measurable indices. It is true that biometric figures are useful as constitutional guides but we can by no means make a constitutional classification solely on the basis of mathematics (Reichert).

No index, no matter how carefully elaborated, is ever an absolute criterion, and as I have stated before there can be little or no consideration of function in such mathematical treatment.

Classification. If we cannot establish a normal, how can we establish and classify groups of individuals? Brugsch takes the position that attempts at classification are relatively useless, that we must study the personality of the individual—the components are so varied and many, with infinite combinations of possibilities of characteristics that we face an impossible task in seeking to assemble individuals into like groups—indeed he sees the essence of constitution in the absolute differentiation of each individual.

No matter how correct in principle, for practical purposes every investigator in the constitutional field has made use of some classification and inasmuch as these investigators have approached constitution from divergent fields of interest, each has made use of criteria which were of chief interest to him.

Hackel has enumerated some of these more important methods of classification:

1. External form and body mass—(Aschner, Weidenreich, Brugsch, Manouvrier, de Giovanni, MacAuliffe, Stockard, Viola, Kramarenko) or anthropometric profile (Newsky, Schewkunenko, Breitmann, Draper).

2. The proportionate size of various organs and organ systems—(Rokitansky, Beneke, Viola).

3. The preponderance of certain anatomical or physiological systems—(Hippocrates, Sigaud, de Giovanni).

4. The relation of the connective tissue elements. In the following groups certain physiological concepts are brought into the picture—(Krylow, Beneke, Bogomolez).

5. A partial congenital deficiency of weakness or the wear and tear of certain organs or mesenchymal tissues—(Martius), (Pfaundler, Stoerk).

6. Fundamental functional differences—(Wunderlich, de Giovanni, Stiller).

7. Muscular tone—(Tandler).

8. Functional capacity of the organism—(Günter, Tschernorutzky).

9. The rapidity and energy of motion—(Perez, Kramarenko).

10. The preponderance of anabolism or catabolism—(Pende, Foillee).

11. The correlation of the total organism and the hypo- or hyperfunction of the glands of internal secretion—(Paltauf, Jul. Bauer, Mathes).

12. The preponderance of the parasympathetic or sympathetic nervous system—the preponderance of conscious acts—(Eppinger and Hess, Borchardt) (Leshaft).

13. General biological or genetic concepts—(Bean, Bryant, Bartel, Bogomolez, Hart, Mathes, Bunak).

14. The concept of predominant disposition to various diseases or the diminished resistance of certain somatic types against a particular disease—(Hippocrates, de Giovanni, Rokitansky, Gowers, Beneke, Viola, Bartel, Kretschmer, Hart).

In these classifications there are many overlapping concepts. Certain terms such as long type, leptosome, narrow chested, microscler, graceful, linear, longilineus, dolichomorphic, are synonymous and characterize similar constitutional types.

Bipolar classification: From the practical aspect classifications may be extremely simple, indeed, apparently the simpler, the more useful.

Hippocrates differentiated a number of types, outstanding was the asthenic and its opposite, the sthenic or muscular-athletic type.

At the one pole the Hippocratic school placed the long, thin, flaccid individual—presumably predisposed to tuberculosis—at the opposite extreme the "apoplectic" type—florid, stocky, strong. The former type built mechanically to overcome distance, the latter, mass. The former in general, quick, alert but with little reserve; the latter, slow, ponderous, enduring.

It is interesting to observe that we have not moved far from that simple classification despite our modern science. When Stiller described his "asthenic" he did it in Hippocratic terms and associated with the type a definite lack of resistance to tuberculosis. The individual had a delicate framework, weak and deficient musculature, little fat or connective tissue, a long chest and low diaphragm, a small heart and vascular system.

The French and English supplied the opposite type in arthritismus and gouty diathesis—and with this latter group (the sthenic or typus apoplecticus) there has been associated a liability to diabetes, gout, concretions and arteriosclerosis (wear and tear disease in general) sensitization (asthma, hay fever, urticaria, migraine) and a number of skin diseases, particularly psoriasis.

While the term asthenic has been retained the term pyknic for the heavy individual has come into more common usage. It is obvious, of course, that these terms cover a wide latitude. The extremes, asthenic and pyknic, make up approximately 10 to 15 per cent at the opposite poles of the human form, and the intermediary zone is naturally made up of alloys of every degree, including the so-called athletic type.

This bipolar grouping which Stockard in particular has emphasized is not only biologically sound but clinically useful and forms the basis for most of the popular classifications.

The Slender Type (leptosome, longitudinal, linear, dolichomorphic; asthenia as an extreme variant)	*The Broad Type (plethoric, apoplectic, pyknic, gouty, "arthritismus," the round type, brachymorphic)*
The individual is long	The individual is broad
Extremities are long	Extremities are short
The face is narrow, oval; the nasal bridge is narrow	The face is broad, round; the nasal bridge is broad
The neck is long	The neck is short
The chest is long and flat, the costovertebral angle is small, the intercostal spaces are wide	The chest is barrel shaped, the costovertebral angle is larger, the intercostal space is narrower

The diaphragm is low	The diaphragm is high
Dolichocephalic	Brachycephalic
Poor connective tissue reactivity	Pronounced connective tissue reactivity (fibrous)
Bony framework is delicate	Bony framework is strong
Skin is thin	Skin is heavy and coarse
Hair is fine, usually well preserved	Hair is coarse and abundant, frequently lost early
The tissue storage is deficient (few reserves)	The tissue storage is pronounced, (reserves good, especially fat, glycogen, etc.)
The heart is small (hypertrophy poor)	The heart is large (hypertrophy good)
The arterial system is narrow	The arterial system is large
The superficial capillaries not marked	The superficial capillaries dilated
The viscera are mobile	The viscera are well fixed
Visceroptosis, varices, intussusception, prolapses and hernias common	Rare
Bodily circumferences small.	Bodily circumferences large.
Smooth muscles hypotonic	Smooth muscles hypertonic
Sluggish general reactions except nervous and vasomotor systems	Hyperirritability in childhood; sluggish in later life
	Thyroid activity lessened
The thyroid activity may be increased	Psychic manifestations—cyclothymic
Psychic classifications—schizothymic	Larynx small, voice, tenor
Larynx large, voice, barytone	Near sighted
Far sighted	Mechanical adaptation to overcome mass
Mechanical adaptation to overcome distance and time	Disease disposition—early exudative diathesis, late wear and tear diseases, metabolic diseases (gout, diabetes, concretions, sensitizations, etc.)
Disease disposition—tuberculosis	
Moderate lymphatic reaction	Pronounced lymphatic reaction

We may accept these opposite and extreme types as characteristic biological variants from the normal type. As we shall see later the habitus is hereditary and distinctly associated with the individuality i.e., the personality of the organism. We must immediately correct the assumption that habitus being hereditary, and habitus being associated with certain disease dispositions, the genotype is of sole importance and therefore therapeutic correction impossible. The habitus of the individual may in part at least be the result of altered functional reactivity.

CONSTITUTIONAL ALTERATION OF BODILY FUNCTION. What is the fundamental difference in these biological variants that is to account

for their difference in their relation to infectious or metabolic disease? Stiller sought the answer in a general deficiency of the asthenic. But actually there is no such thing as general deficiency. Organ deficiencies are always compensated in some fashion and the slender individual is not necessarily a weak individual—he may be wiry and tough. Tandler seeks the difference in tonicity—the flaccid muscle or connective tissue fiber in contrast to the tonic quality of the pyknic. Bouchard sought the key to the pyknic habitus and arthritismus in a slowing of tissue reactivity, a bradytrophy.

But if we are dealing with reactivity, it immediately becomes apparent that we must examine physiological reactions and more particularly the autonomic system, that is, the chemistry of the body, the hormones and the vegetative nervous apparatus.

Among others Tschernorutzky and his pupils have made studies in this direction. Tschernorutzky very correctly assumes that the constitution of an organism is the sum total of the morphology and functional activity, is therefore fully analogous to the functional constitution of a molecule or a cell. He uses the classification of asthenic and hypersthenic or pyknic and his intermediary or third type, normosthenic, corresponds to the muscular type of Kretschmer.

Using the Pignet index, the latitude for the normosthenic was found to be between +10 and +30, for the hypersthenic type below +10 and for the asthenic type over +30.

I take the following from his correlation of habitus and function:

Muscular strength. In males the correlation between absolute muscular strength and the length is closest, in women the closest correlation is with the weight.

Blood pressure. The height of the blood pressure diminishes as we go from the hypersthenic to the asthenic group.

Respiration. There is greater vital capacity among the asthenic types, i.e., the asthenic is in a more favorable position as far as gaseous exchange is concerned than the hypersthenic.

Hematopoietic system. There is greater functional capacity and a higher red count in the hypersthenic. Asthenics show greater tendency to anemia. Bryant (using the classification of herbivorous for the pyknic and carnivorous for the asthenic) as well as Liwschin find similar correlations.

Metabolism—carbohydrate. Diastatic enzymes of the blood are lowest in hypersthenics, higher in asthenics—the latter, too, show a tendency to hyperglycemia.

Sugar tolerance test—The asthenic is more labile; the sugar goes up and returns to normal more rapidly.

Fat metabolism. The lipolytic index is greater in hypersthenics. They produce, use and store fat to a greater extent. There is increased lipemia and lipolytic energy.

It is, of course, well known that the distribution of fat is not only under the influence of the endocrine glands but distinctly constitutional, as in lipophilia and lipodystrophy. The quality of the fat itself differs. In adipositas, for instance, fat contains less water and is more fluid than is the fat of relatively slender individuals.

Lipoids. Miasnikow, Tschernorutzky, Glinka-Tschernorutzkaja and Jackson have shown that there is a distinct correlation between cholesteremia and constitution. Miasnikow found that hypersthenics had an average of 1.8 per cent, the asthenic of 1.3 per cent. Tschernorutzky finds similar results.

While the results are somewhat altered by age, this increase is characteristic for all constitutional types. The constitutional alterations in cholesterol are therefore not altered by sex, age or disease.

Uric acid. Grotell found an increase in the uric acid in hypersthenic individuals and of course an increase in the individuals with associated pathological conditions (obesity, gout, gall stone disease, arteriosclerosis, hypertonia, bronchial asthma).

Ferment balance. The catalytic energy of the blood is maximum with hypersthenics and lowest with asthenics. Morew found the same thing with children. Summarizing, one can say that hypersthenics show high catalytic and low diastatic energy.

Bilirubin. The asthenics show higher bilirubin values than the hypersthenic.

Basal metabolic rate. The difference in the basal metabolic rate between the slender and the broad type, a phenomenon which Borchardt and others have observed and which forms part of the bradytrophie of the pyknic, probably finds its explanation in the work of Bohnenkamp. Bohnenkamp observed the greater degree of peripheral insulation in the female as compared to the male organism, with distinctly lower skin temperature as a result, and with correspondingly greater conservation of energy and a lower metabolic rate. The pyknic, with peripheral fat storage, is in a similar condition as contrasted to the asthenic—the metabolic rate is adjusted to the better insulation of the pyknic.

We may assume that fat and reserve energy in general has a greater importance in the female organism because of the reproductive needs

and the process seems specifically inherent in the female endocrine balance. If this assumption is correct then the pyknic constitution in the male is the bridge toward femininism and the asthenic type in the female, the bridge toward masculinity. In the psychic sphere this is occasionally quite apparent.

Levinson and I made studies on a series of 100 so-called normal men whom we have analyzed on the basis of the Weight / Length ratios (weight in pounds—length in inches). The average for the group of 100 men of all ages was 2.17. We have contrasted the group of 25 of the most slender individuals (ratio 1.87) and of 25 of the heaviest (ratio 2.32) insofar as chemical and functional differences appeared. The average age for both groups was approximately the same.

	HEAVY GROUP	SLENDER GROUP
Capillaries	0.57	0.63
Blood sugar	73.8 mgm.	69.5 mgm.
K/Ca ratio	2.08	1.81
Muscular irritability	3.48 m.v. (threshold)	2.38 m.v.
Epinephrine reaction	+8% increase	+12% increase
Cholesterol	225 mgm.	210 mgm.
Reaction from Kromayer irradiation	2 hours	2.2 hours
CO_2 Combining power	54.4	58.5

When we now add clinical patients to such a list (a total of 175 patients and so-called normal individuals) and examine the 20 individuals at the extreme ends of the scale, the slender group has a Weight/Length ratio from 1.43 to 1.76, the heavy group from 2.47 to 3.17.

In the slender group we find individuals with disturbances of the vasomotor system (Raynaud's disease, glaucoma, urticaria, ulcer, nervous individuals, etc.); in the heavy group, the cardiovascular-renal diseases predominate.

In the slender group the K / Ca ratio varies from 1.24 to 3.1, in the heavy group from 1.45 to 3.22, the averages being 1.89 and 2.06 respectively, again emphasizing the lower ratio of the slender type.

When examined by means of the epinephrine reaction the heavy group was predominantly vagotonic as compared to the slender group. In the heavy group only four showed an increase in systolic blood pressure as compared to ten in the slender group after epinephrine injection.

In another study limited largely to the capillary permeability, Willis and I examined some 66 normal medical students. Of the group 9 were

university athletes. With one exception they represented powerful, broad types; there were no asthenic types among them. Again we see the same lowering of permeability with a distinct functional difference. The average capillary permeability of the entire group of 66 was 68; for the athletic group the average was 62.4.

I cannot enter into detail regarding the associated observations concerning differences in the physiognomy and the superficial structure of the body (the skin, hair, vasomotor phenomena, texture, sweat and sebaceous glands, etc.) but the association of superficial dilatation in the pyknic and the peripheral contraction of the skin vessels in the asthenic with its resulting pallor, is so characteristic that it deserves emphasis.

Otfried Müller and his associates have in recent years described the superficial vessels of various constitutional types in considerable detail. Concerning the asthenic he summarizes as follows:

"Here are the individuals with tense arterial neuro-vascular mechanism, with the pseudo-anemic pallor of the face, the bluish, clammy moist hands, with dilated subpapillary venous plexus."

On the other hand Otfried Müller observed a different set of vessels in cyclothymic-pyknic individuals—the short, fat individual—here the red face is associated with large capillary venous dilatations, especially over the nose and cheeks. Here are wide capillaries in the upper chest wall with venectasia in the region of the pelvis and the lower extremities. Generally speaking the capillary bed of the extremities shows less change than in the asthenic.

Stefko ascribes the chief rôle in this development of the peripheral capillary plexus to the thyroid.

It is important to keep in mind the integration of form and function. In the case of the athletic type one has increased function resulting in proper growth of organs and bones, etc. In the pyknic type, as we shall see later, we have dysfunction of certain organs. Frequently starting out with an "irritable constitution" that finds expression in inflammatory or exudative diathesis, lymphatism, etc., we later find eosinophilia and anaphylactic diathesis, vagotonia, etc.

Why should the early irritable constitution lead to the broad growth? Is it due to irritability of the mucous membranes of the upper respiratory tract? Is it general irritability associated with good functional response, abundant blood supply, with the resulting hypertrophy of connective tissue elements?

Here the intensity of reaction must be considered. If an organ is relatively irresponsive then minor irritants remain without effect on

the cells and we have the appearance occasionally of the atrophy of disuse. On the other hand we may have a lowering of the threshold for irritants. In youth under those conditions we may have hypertrophy if the nutritional condition is able to keep up with the activity. Under all conditions we find early wear and tear.

According to Brugsch, the chief factor in producing muscular hypertrophy lies in the blood supply. If the vascular system is small, we get no hypertrophy.

The intensity of reaction is predicated on the capacity of the cell as well as on the strength of the irritant. From the constitutional point of view this reactivity to stimuli is of utmost importance. If the threshold is lower, then the irritability results in increased activity and ultimately leads to greater wear and tear. Here again alteration in reactivity (sensitization) plays a rôle.

We shall now proceed to examine the differences from another angle. An entirely novel inter-relationship of the pyknic type (arthritismus) was disclosed when Comby showed that the commonly observed diathesis of childhood, that is, the exudative diathesis and lymphatism were manifestations of arthritismus in childhood. Now there has been reason to believe heretofore that the metabolism of the pyknic is relatively sluggish and the general reactivity lessened. On the other hand, in exudative diathesis and lymphatism we have no indications that we are dealing with sluggish organisms.

Obviously, too, the nervous irritability, the increased antibody production, the tendency to asthma, and the frequent anaphylactic phenomena needed some other explanation.

In addition to the differences in the reactivity of the connective tissue we have no explanation why the asthenic remains slender and pyknics are apt to be fat, nor can we explain the tendency toward stone formation, that is, the precipitation of calcium, uric acid, and cholesterol in the tissues in the pyknic type.

While formerly we assumed that the piling up of these metabolic increments was due to the slower metabolism of the pyknic, we must now consider the possibility that this type of individual holds such substances with tremendous tenacity. Brugsch speaks of a lipogenic tendency of the tissue and Bauer of a lipophilia. Both could show that the tendency to fat deposits and retention was maintained even in hunger. Gudzent spoke of a "üretohistechie" (in gout) and Umber of a histiogenic retention of uric acid with active participation of the joints in the production of uric acid deposits.

This is in line with the fact that uric acid saturation of the blood in leukemia is as a rule not followed by gout. Without doubt blood saturation, colloidal precipitation at the inflammatory focus, and the formation of new surfaces for the precipitation are of importance. But added to this there is a local factor (H. Strauss).

The same phenomenon apparently underlies the formation of concretions and the precipitation of calcium and cholesterol in arteriosclerosis. That is, super-saturation of the solution and the loss of protective colloids through the formation of new surfaces during the course of the inflammatory process. Under all circumstances there is a tendency of the pyknic types to deposit their non-living substances.

Borchardt believes that Umber's term "histiogenic retention" deserves broad application and even ascribes the increased lymph saturation of the tissues in childhood to similar causes.

Now as opposed to this the asthenic is totally different. His tissues are poor in water, the connective tissue is poorly developed, the bones thin, calcium precipitation is very poor.

So then, in addition to the early increased irritability and the adult wear and tear of the pyknic as compared to the deficiency of the asthenic, the histiogenic retention of the pyknic and its failure in the asthenic is a trophic principle probably sufficient to account for the differences in the reactivity of the two types towards diseases.

Katase seeks to explain the fundamental differentiation of the slender and the broad type on a dietary basis. He has found that alkalin diets in young animals are followed by relative broad development, acid forming diets by longitudinal development and believes that diet is to be considered as a factor in the production of such variants in humans. I have found that typically pyknic individuals apparently have a tendency to retain basic valencies as contrasted to the leptosome.

While irrelevant in his connection it may be of interest to mention Borchardt's belief that the recognition of the functional antagonism of the two types may result in a changed point of view of prophylactic-constitutional therapy. Martius repeatedly emphasizes that constitutional variants are not diseases and therefore are not subject to treatment. The point is, however, that when we consider treatment of the individual patient, they always require consideration. After all it is not the bodily form but the anlage that is inherited. Weakness can be overcome by training, hyper-irritability through protection against irritants. Without doubt, nutrition, physical therapy and some drugs

may be able to bring about alterations, in that the histiogenic retention of the pyknic can be counteracted by flooding the body, by depletion, by mineral waters and hydrotherapy. It is an interesting point of view in that we begin to understand some of the empiric therapy of bygone medical practice.

Now it must be evident that when we deal with far reaching differences in the habitus, in physical and psychic reactions, in disease manifestations, as we apparently do in the antipodal types just considered, we may anticipate differences in the glands of internal secretion, as in the autonomic status. The effects of the glands of internal secretion are obvious in the glandular deficiencies and in the dysplasias that we observe in infantilism, gigantism, cretinism, etc. (gonads, pituitary—thyroid, etc.). We have the right to surmise their effects in constitutional differences in normal individuals but the complexity of the problem is limitless and we may very well sympathize with Brugsch's point of view in discarding classification in toto and concentrating on the individual.

We have presented the extreme variants and there seems a logical basis in believing that apart from the habitus the types are oppositely oriented functionally in many ways. But between the poles lies the great bulk of our material, with every possible shading of structure, form, function, alloyed in the course of descent and transmuted in the crucible of adjustment to the environment. I emphasize this in passing lest the preceding discussion leaves the impression of dogmatism.

THE GENOTYPE. It is quite outside of my province to discuss the details of inheritance—mutation—paravariation—hybridization—or the inheritance of acquired characteristics. Naegeli's discussion of the subject is very thorough and written from the medical standpoint and the recent summaries in the American literature give an excellent survey of the field from the point of view of the geneticists.

The geneticists have apparently distinctly refuted the Darwinian concept of evolution as far as it relates to the formation and transmission of useful characteristics. When, however, we deal with such a problem as resistance to tuberculosis, and find that the young female is weeded out from the racial group even before reproduction or early in the reproductive period, then we must take cognizance of the possibility that in the gradual acquisition of resistance to the infection the human race has gone along the pathway of gradually eliminating the susceptible strains.

We have seen that the two antipodal types presumably represent extreme variants of the domesticated human, a genus that epitomizes

extreme hybridization with the consequent appearance of a variety of minor characteristics or groups of characteristics that result in unlimited differentiation.[1] Perhaps it is a result of the confusing bastardization in humans as contrasted to clear-cut experimental genetic study that criticism of the "gene" hypothesis has occasionally made itself apparent, as in a recent paper of Greil.

The human is a poor animal for genetic study; the interval between generations is too long, the control of the characteristics bred utterly impossible. But what we may lack in scientific exactitude we partially compensate in racial experience and in the consciousness of common man the laws of heredity are firmly fixed.

Some clear cut anomalies, certain of the heredo-familial degenerative diseases of the nervous system, some very striking metabolic disturbances such as pentosuria, alkaptonuria, can be definitely followed as gene determined characteristics and Borchardt's table pages 298 and 299 gives some idea of the number of clinical conditions that can be grouped in this regard.

Naegeli presents a number of other mutations in his treatise, including the psychoses, hypertension, urolithiasis, etc.

It is to be remembered in connection with this table that the transmission of many of these congenital defects and maladies may vary. For instance, there are many cases of migraine that appear as dominant, others as recessive in transmission.

Galant assumed that "the recessively inherited characteristic, as, for instance, an inheritable disease which under proper conditions of life may exist merely as a disposition and therefore be latent, becomes active and clinically apparent as soon as exogenous factors bring about an unbalance which accentuates the inherited disposition and from the condition of latency and recessivity of the disease, dominancy develops."

The fact that in humans we deal frequently with the transmission of latent defects or dispositions makes the analysis of the mode of transmission particularly difficult.

A great deal of skepticism has been evident concerning the results of efforts to apply Mendelian laws to observations in clinical pathology.

Haecker, for instance, groups the observations under two categories when he says:

> On the one hand those cases in which the disease occurs in a family with great similarity as far as localization, period of appearance and the clinical course is

[1] The work of Fischer on Hottentott-Boer bastards is of particular interest.

DOMINANT	RECESSIVE	SEX LINKED RECESSIVE
	I. Sense organs	
Hemeralopia	Day blindness (total color blindness)	Atrophy of the retina
Myopia	Retinitis pigmentosa	Red-green blindness
Hyperopia	Albinism	Congenital optical atrophy
Distichia	Congenital deafmutism	Albinism with nystagmus
Congenital ophthalmoplegia	Otosclerosis	Night blindness with myopia
Coloboma of the optic nerve		
Coloboma of the iris		
Irideremia		
Ectopia of the lens		
Congenital juvenile cataract		
Glaucoma		
Congenital nystagmus		
Ptosis		
Astigmatism		
Labyrinthine deafness		
	II. Nervous system	
Congenital spastic spinal-paralysis	Friedreich's ataxia	Progressive neurot. muscular atrophy
Congenital cerebral ataxia	Myoclonic epilepsy	
Progressive muscular atrophy	Amaurotic family idiocy	
Huntington's chorea	Myopathic muscular atrophy	
Thomsen's disease	Spinal muscular atrophy	
Familial periodic myoplegia	Progressive spastic paraplegia	
Congenital tremor		
Neurofibromatosis		
Angioneurotic edema		
Migraine		
	III. Skin	
Epidermolysis bullosa simpl.	Albinism	Bullous dystrophy
Keratosis palm. et. plant	Epidermolysis bullosa dystrophica	Anidrosis with hypotrichosis
Porokeratosis	Xeroderma pigmentosum	Keratosis follicularis (Typ. Lameris-Siemens)
Atheroma	Ichthyosis congenita	Localized albinism
Hypotrichosis	Hydroa aestivale	

DOMINANT	RECESSIVE	SEX LINKED RECESSIVE
	III. Skin—*Concluded*	
Hyperkeratosis unguinum		
Anonychia u. leuconychia		
Freckles		
Hyperidrosis		
Xanthomatosis		
Mult. telangiectasis		
Cong. chron. trophedema		
Ichthyosis vulgaris		
	IV. Miscellaneous	
Cystinuria	Chondralloplasia	
Fam. hypospadia	Microcephaly	
Eunuchoidism	Cong. hip disease	
Cong. diabetes insipidus	Club foot	
Polydactylism	Alkaptonuria	
Syndactylism		
Brachydactylia		
Ankylosis finger joints		
Mult. cartilaginous exostoses		
Dupuytren contracture		
Osteopsathyrosis		
Hairlip		
Prognathism		
Splitfoot		
Hallux valgus		
Funnel-chest		
Asthenia		
Hemolytic fam. icterus		

concerned and occurs in a very definite manner either continuously through several generations or apparently not transmitted, but among several brothers or sisters. Without doubt one would get the impression that we are dealing here with something that is associated with Mendelian transmission. On the other hand there are cases known where undoubtedly an hereditary basis exists, but in which the clinical picture of the disease may show marked variation in different individuals or an inter-familial alternation of various diseases in an identical localization as, for instance, in the stomach or the kidney or the liver. What seems to be inherited here is obviously a constitution or a certain habitus or status—a soil on which varying pathological conditions may develop depending on conditions.

Albinism affords an excellent example. This may occur as a definite Mendelian recessive characteristic, but in other cases as an independent, more or less general degenerative anomaly, and associated in an irregular fashion with other anomalies (the so-called status albinoticus of Reynold).

Clear-cut Mendelian inheritance we find apparent only in those characteristics which in their developmental history are due to relatively simple ontogenetic processes and can develop more or less independently of other characteristics.

The descent of the individual characteristics can best be followed when we are dealing with anomalies or abnormalities, particularly the abnormalities of metabolism. Carbohydrate metabolism has offered interesting sidelights.

In humans pentosuria shows a dominant inheritance. That is, there is a direct inheritance of the children from the parents without the skipping of one or more generations. If an individual in whose ancestors pentosuria occurred is free from this condition, then the descendants of that individual will also be free, provided, of course, that the partner is free. Of course this rule is not without its exception. Even in case of dominant inheritance a generation may sometimes be skipped without the cause being known. In recessive inheritance as, for instance, retinitis pigmentosa, the number of involved descendants is materially lessened. Latency of the condition is a rather frequent phenomenon and transmission to descendants under these circumstances from apparently normal ancestors, is common.

But if peculiar chemical and physiological anomalies are transmissible, the transmission of normal chemical—hormone—and autonomic patterns of reactivity must also, logically, occur and the determination of the mode of inheritance becomes of immediate interest because of the importance of functional and chemical reactions of the body in relation to disease.

The problem has been attacked by studies in unioval and bioval twins and these studies have yielded considerable information but, of course, the results cannot be compared as to certainty and comprehensiveness with those obtained in animal experimentation. Siemens (1924) in his work on the pathology of twins cites instances of striking concurrence of pathological reactivity in twins, presumably due to reactivity of the autonomic apparatus upon which the clinical course and the outcome of so many of the infectious diseases depend, as Czerny, for instance, has emphasized.

Geyer as well as Curtius and Korkhaus have recently presented pertinent studies of this nature. Making use of the reaction of the vasomotor system to epinephrine and of the Volhard test (determination of urine secretion and blood hemoglobin after standard quantities of water have been taken on an empty stomach) they found that unioval twins showed marked similarity. In one pair of twins examined by Curtius and Korkhaus identical extra-systoles occurred, indicating that the identical stimulus affected both the vagus as well as the heart ganglia in an identical manner. The Volhard water test showed an absolute concordance in unioval twins, indicating a remarkable parallelism of the reactivity of the water exchange. It has previously been shown that there may be an inheritance of the respiratory rhythm, characteristic pulse frequency and blood pressure (Siemens, von Verschuer, Lenz, Weitz, etc.).

Löwenstein found a marked similarity of the reaction of the pupil to light in unioval twins in contradistinction to bioval twins.

Because of the marked similarity shown in the reaction of the skin in unioval twins to pathological influences Curtius and Korkhaus present a series of pathological reactions as evidence of a very deep-seated morphologic as well as autonomic and humoral similarity, the morphologic similarity extending even to the papillary lines, to pigment distribution, as well as vascularization of the skin. As far as the humoral identity of the skin of twins is concerned K. H. Bauer has presented very beautiful experiments with transplantation in unioval twins.

In a tuberculosis of a unioval pair of twins Curtius and Korkhaus found a marked parallelism in the clinical phenomena, not only involving a similarly localized keratosis but a complete parallelism of the lung, lymph gland and tuberculin findings. Levinson and I had the opportunity of studying a similar pair of unioval twins with tuberculosis. Infection apparently took place in early childhood, activation commenced in both between the 18th and 19th year, the clinical findings and the clinical course were identical.

Ganther and Rominger have shown similar concordance in exudative diathesis.

Bernheim-Karrer has examined the bactericidal property of serum of unioval twins and has found certain decided differences, and Westergreen has studied the sedimentation reaction. He found a marked parallelism in unioval twins. On the other hand Curtius and Korkhaus found distinct differences in the Moro Reaction in different unioval twins. The same holds true, too, for the vaccine reaction.

Particularly in scarlet fever do we find marked changes in the temporary disposition but it is hard to say when this dispositional change takes place in unioval twins. Possibly even intra-uterine influences may play a rôle. Thus Schroeder observed a pair of twins where only one partner was diseased with small pox pustules, and Curtius and Korkhaus agree with Verschuer that the constitutional (genotypic) differences of an individual do show marked alterations as a result of paratypical factors.

In all studies of twins it must be kept in mind that occasionally heterozygotic twins may have a single membrane and on the other hand that monozygotic twins may develop with two separate membranes. The intra-uterine development of twins may, of course, vary considerably particularly in the amount of foodstuffs received by one or the other.

Environment. If the genophase offers unlimited possibility of variation, we face a field that is just as extensive when we turn to factors that influence the individual organism in utero and after birth. The range of environmental adaptation of even the most primitive cell is relatively wide, although the mechanism of response may be rather simple; but as we advance in the scale of animal life the range of adaptation becomes even greater as the mechanism and the means of adjustment become more complex.

But somatic adjustment to environment must fundamentally mean biophysical or biochemical alteration of cell structure and function. The modification may be wholly reversible, it may be partially reversible, we may merely develop somatic memory, probably the result depends on the state of the cell, on the development of the receptor mechanism and the intensity or duration of the stimulus.

When we deal with human conditions the modification of the individual by environment becomes extraordinarily complex because of the interdependence of the vegetative functions, hormones, the intellectual activity and the emotional life.

Interestingly enough, the cell at the time of fertilization is peculiarly susceptible to environmental influences. The cell is also vulnerable at the time of mitosis but naturally an injurious modification brought about at the time of fertilization is of greater importance than later influences on individual mitotic divisions because of the fact that every descendant cell from the time of fertilization is involved.

At fertilization the cell membrane becomes much more permeable. It is then more susceptible to poisons, it has a low electrical resistance, offers lessened resistance to the passage of hydroxyl ions in hyper-

alkaline sea water, cytolysis occurs much more readily with hypotonic salt solutions, etc. (Spek).

This is of importance in humans because of the well authenticated effect of acute alcoholism on the embryo when fertilization has occurred during acute intoxication. In animal experimentation interesting confirmatory evidence has been produced. Other intoxications very likely have a similar effect (lead, narcotics, bacterial toxins, etc.) but after fertilization the young embryo is relatively non-susceptible.

Nevertheless physical and chemical alterations of the milieu are still of importance. For instance, it is very probable that a change in the calcium and potassium content of the fluids may lead to lessened or increased cohesion of the cells and their membranes with possibilities of resulting distortion, fusion or duplication of organs or parts of organs. The specific defects so created are, of course, not transmissible, although the general tendency toward the formation of such defects may be.

After birth the individual becomes subject to a far greater number of influencing factors. Stimuli resulting from changes in nutrition, temperature, composition of the air, care, etc., all play a very marked rôle, nutrition in particular being the most important factor quantitatively, as well as qualitatively.

Among the exogenous stimuli, the effectiveness of which depends to a certain extent on duration as well as intensity, trauma—physical as well as psychic—exertion and the effect of work, light, infections, intoxication, alcohol, narcotics, industrial poisons, might be mentioned—all become evident not only in the normal organism but particularly in the individual with pre-existing disease. Endogenous changes, cyclic in character, as, for instance, menstruation, the menopause, the circulation of abnormal metabolic products, cachexia, all affect constitutional reactions most profoundly while the absence of normal stimuli makes itself felt especially in the blood vascular system and on the endocrine activity. It is interesting to recall that during the war in Europe, with the population under intense emotional and physical strain, exophthalmic goitre actually decreased, the increase in nervous strain being more than offset by the lowering of thyroid activity due to food deficiency. Adrenal deficiency as well as deficiency of other glands of internal secretion was not only clinically evident, but demonstrable at autopsy. The vitamine deficiencies have an effect not only in producing disease per se (scurvy, beriberi, etc.) but in lowering resistance to infections.

There are profound constitutional effects following recovery from disease, either in the form of increased or diminished susceptibility to

infection, or of increased or diminished reaction to a pre-existing infection or organ dysfunction. Here belong, too, the results of repeated sub-clinical infections and the localization of bacterial infections or of toxic manifestations in remote organs which have become susceptible because of previous injury.

To all must be added the influence of social organization, family and race, religion, economic and occupational position, and finally perhaps, the most fundamental of all, the cosmic influences. Long before the advent of life on the planet cosmic forces have exerted effects in definite cycles—the diurnal rhythm from maximum solar effect to its complete absence—the lunar rhythm and the complete annual rhythm of the seasons has left its imprint on almost every biological process. It may be of interest to note that Brugsch includes this fundamental effect of rhythm, constant adjustment of reaction to conditions varying between opposite poles, in a definition of constitution in this fashion:

> Constitution is the unity of physical-chemical elements making up a definitely definable vital system whose co-ordinating internal forces establish (by means of wave-like fluctuations) a balance with external forces (cosmic, environmental, etc.) whereby the internal forces are determined and laid down in the genes but the final stamp is placed on the constitution by reactivity to the environment.

At the beginning of this review I called attention to the fact that in addition to tuberculosis, the "wear and tear" diseases, cancer and the psychoses loom larger and larger in the medical interest as the infectious diseases are conquered. It is in this group that constitution is of particular importance.

Freedom from clinical tuberculosis depends on natural resistance and not on the immunity. There appears to be no escape from this conclusion despite the recent work of Calmette. From the time of Beneke's careful study (1881) to the recent papers of Saltykow, Hackel and Stefko, pathological anatomists have demonstrated the peculiar liability of the slender individual; Neuer and Feldweg among others have demonstrated the same fact in clinical studies; Pearl, Gottstein and Lange have presented the statistical analyses. The painstaking familial studies of Alons, Doyer and Münter but confirm these conclusions. This natural resistance is, of course, not only genotypic, it is clearly influenced by the paraphase—and here the observations on the influence of menstruation, diet, season, the psychic status, are so obvious and convincing that they can no longer be questioned. Constitution, genotypic and paratypic, may therefore even be the determining factor in an infectious disease such as tuberculosis.

That heredity is of importance in tumor disposition has been established largely by American investigators (Loeb, Slye, etc.). Here we presumably deal with two factors, a disposition to blastoma formation on the one hand, and inheritable resistance on the other. But exogenous elements frequently enter into the initiation of the process.

In psychoses we deal with purely endogenous factors and Kretschmer's work, now confirmed by many investigators, has definitely associated the extreme variants—the slender and the broad individual with Kraepelin's two fundamentally distinct psychoses, the schizophrenic and the maniac depressive. The evidence that has accumulated in the latter field is particularly convincing because it has been based on human material and because we have no important exogenous factors in question.

We are justified in drawing the conclusion that fundamental differences in the hormone and chemical constitution of the individual find expression not only in pathological but in physiological and psychic reactions of utmost importance. The complexity of the problem, however, makes definite progress slow when we actually face clinical application. Nevertheless such advances which we can record give a scientific basis for observations that go back to our oldest clinical sources.

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